Ion Exchangers in Food Processes[a]

A. B. MINDLER

The Permutit Company, New York, New York

Several processes in which ion exchange materials are applicable to the food industry are briefly discussed. A few limitations of the method are listed.

The food industry has found ion exchange processes very helpful in the past both for water conditioning and for numerous applications outside water treatment. These processes may be summarized as follows:

1. Removal of undesirable electrolytes from solution
2. Addition of specific ions to solution
3. Separation of electrolytes in solution
4. Recovery of valuable electrolytes from solution
5. Catalysis

It is the purpose of this discussion to illustrate how these ion exchange processes have been used by others in the food industry and to investigate some of the limitations involved.

History

The phenomenon of ion exchange was discovered in about 1850 by H. S. Thompson and J. T. Way *(23)*, agricultural chemists in England, during some work on soils. For about 50 years thereafter, no industrial use was suggested for ion exchange. In 1896, Harm *(7)* disclosed the use of a calcium zeolite for removing the alkali metal cations, sodium and potassium, from sugar beet juice in order to reduce the amount of molasses formed during sugar manufacture. Rumpler *(18)*, Gans *(5)* and Bachler *(1)* also used calcium zeolites for improving sugar yield during the succeeding 30 years. The introduction of another ion exchange process, the demineralizing process, in 1936, resulted in continued investigations of ion exchange in this problem of long standing.

Materials

Ion exchange may be defined as the "reversible interchange of ions between a liquid phase and a solid phase which does not involve any radical change in the solid structure." Ion exchange materials, as commercially produced, are generally granular solids having a grain size between 10 and 60 mesh. (U. S. Bureau of Standards). There are two different types of ion exchange materials, cation exchangers and anion exchangers. These in turn may be broken down into several broad classifications:

A. Cation Exchange Materials
   1. Inorganic cation exchangers
      a. Processed naturally occurring minerals such as the glauconites or greensands
      b. Synthetic inorganics such as the precipitated sodium aluminosilicates
   2. Organic cation exchangers
      a. Processed naturally occurring materials, such as the sulphonated coals
      b. Synthetic resin cation exchangers
         (1) Modified phenol formaldehyde resins
         (2) Sulphonated hydrocarbons
         (3) Carboxylic acid resins

B. Anion Exchange Materials
   1. Aromatic amine resins
   2. Aliphatic amine resins

The ion exchange materials classified above each have specific properties. Every application for ion exchange requires certain characteristics of the materials to be used and therefore the choice of the proper type of ion exchange material is extremely important.

Types of Reactions

There are four different types of ion exchange reactions:

1. Base Exchange, using calcium as an example

$$Na_2Z + Ca^{++} \underset{\text{regeneration}}{\overset{\text{reaction}}{\rightleftharpoons}} CaZ + 2\,Na^{+}$$

where Na_2Z represents the cation exchanger in the sodium exchange cycle

2. Hydrogen Exchange

$$H_2Z + Ca^{++} \underset{\text{regeneration}}{\overset{\text{reaction}}{\rightleftharpoons}} CaZ + 2H^{+}$$

where H_2Z represents the cation exchanger in the hydrogen exchange cycle

3. Acid Removal, using hydrochloric acid as an example

a) $R_3N + HCl \xrightarrow{\text{reaction}} 2(R_3NH)Cl$

b) $2(R_3NH)Cl + Na_2CO_3 \xrightarrow{\text{regeneration}} 2R_3N + 2NaCl + H_2CO_3$ where R_3N represents the alkali regenerated anion exchanger

4. Anion Exchange

$$2(R_3NH)Cl + SO_4^{--} \underset{\text{regeneration}}{\overset{\text{reaction}}{\rightleftharpoons}} (R_3NH)_2SO_4 + 2Cl^{-}$$

The exchange of anions is sometimes used in special applications work, but seldom in water work. In this reaction the salt of an anion exchanger (Reaction 3a) is used.

For carrying out base exchange, either the organic or inorganic cation exchangers may be used. Generally the cation exchanger is in the sodium exchange cycle,

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i.e. regenerated with sodium chloride brine, although any sodium salt may be used. Likewise, the base exchange reaction may be carried out with any other base metal cation in the exchange position by using the proper salt for regenerating the cation exchanger. For carrying out hydrogen exchange, however, only the organic types of cation exchangers are used because the inorganic exchangers are not sufficiently stable to the low pH of acid regeneration.

In the selection of good ion exchange materials, too much attention is frequently given to the capacity of the material. While high capacity is an important characteristic, there are other factors which are even more important. Stability or the ability of the exchange material to continue giving its original capacity, lack of color throwing, rate of reaction, completeness of reaction, and economy of operation, are some of the other important characteristics.

Types of Processes

There are five types of processes in which the reactions of ion exchangers given above may be utilized *(19)*. These are:

a. Removal of undesirable ionic impurities from solution.
b. Addition of specific ions to solution.
c. Recovery of valuable substances from solutions.
d. Separation of electrolytes in solution.
e. Use of ion exchange materials as catalysts or solid contact agents.

Removal of Undesirable Ionic Impurities

Most of the work on ion exchange in the food industry has been conducted on this proces. The largest use of ion exchange in the food industry and indeed in any industry for the removal of ionic impurities in solution is in the field of water treatment. A diagram of a modern Zeolite water softener is shown in Figure 1. The use of ion exchange for treating waters for boiler water makeup, cooling waters, washing solutions and rinse waters, and general process uses have been widely discussed in the literature. However, it might be well

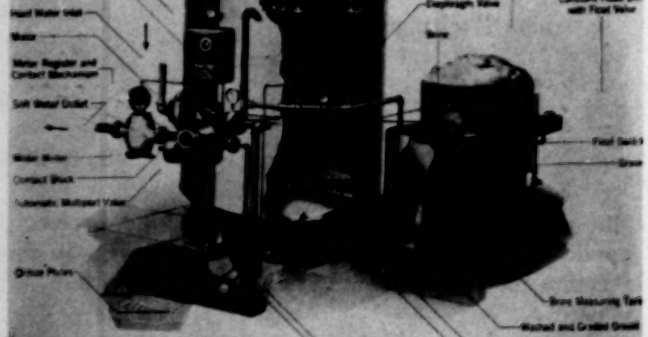


FIG. 1. Industrial Zeolite Water Softener.

to summarize here the importance of using water of the proper quality as ingredient in foods.

During recent years the distilling industry has found that demineralized water is just as good and in some respects better than distilled water, formerly used for deproofing alcohol. The cost of demineralized water is only a small fraction of that for distilled water. The soft drink industry has tightened its standards on the quality of conditioned water used in its products and in some cases ion exchange is used to produce suitable water.

In the canning industry, product quality improvement has also been realized by using properly conditioned water. For example, hard water in canning asparagus causes the buds to open whereas soft water keeps the buds tightly closed. The use of soft water in canning peas is extremely important because this vegetable has a considerable water softening capacity itself. The cellulose structure in the pea is said to take up the calcium and magnesium from the water in much the same manner as a conventional cation exchange material, the canned peas becoming tough and unpalatable. For this reason hardness is especially undesirable in blanching peas.

Base Exchange

The treatment of milk and milk products by base exchange has been used for altering the calcium and sodium content *(11)*. By exchanging sodium ion from an ion exchanger for calcium ions in milk, soft, easily digested curds are formed in the stomach rather than the hard tough curds often formed with cows' milk. This "softening" process has also been used to decrease the browning effects often noted in evaporated milk *(9)*.

Another promising use for base exchange in the alteration of the cation content of a solution is in the treatment of grape juice. By substituting some sodium for potassium and hydrogen in grape juice, it is possible to check the precipitation of potassium acid tartrate, which necessitates the extended storage of grape juice prior to bottling.

Base exchange has been found effective for the removal of heavy metals, for example lead from maple syrup *(24)*. The lead in maple syrup is introduced from the solder used in repairing evaporators and collecting pails. A calcium zeolite was found effective for removing 97% of the lead, reducing it below 1 p.p.m. (dry basis) without affecting the flavor of the maple syrup or changing the ash content.

Hydrogen Exchange

A unique process involving hydrogen exchange for the removal of undesirable impurities from solution has been in commercial use in the manufacture of pectin from grapefruit peel since 1941 *(14, 2)*. This is one of the few commercial applications where ion exchange materials are utilized in a batch-wise process not in a columnar operation. Figure 2 is a flow diagram of this process. The grapefruit peel is ground to particles approximately ¼" diameter which are then washed three times with hot water. The washed peel is mixed with water and Zeo-Karb, a sulfonated coal and extracted for an hour at 195° F. The hydrogen from the

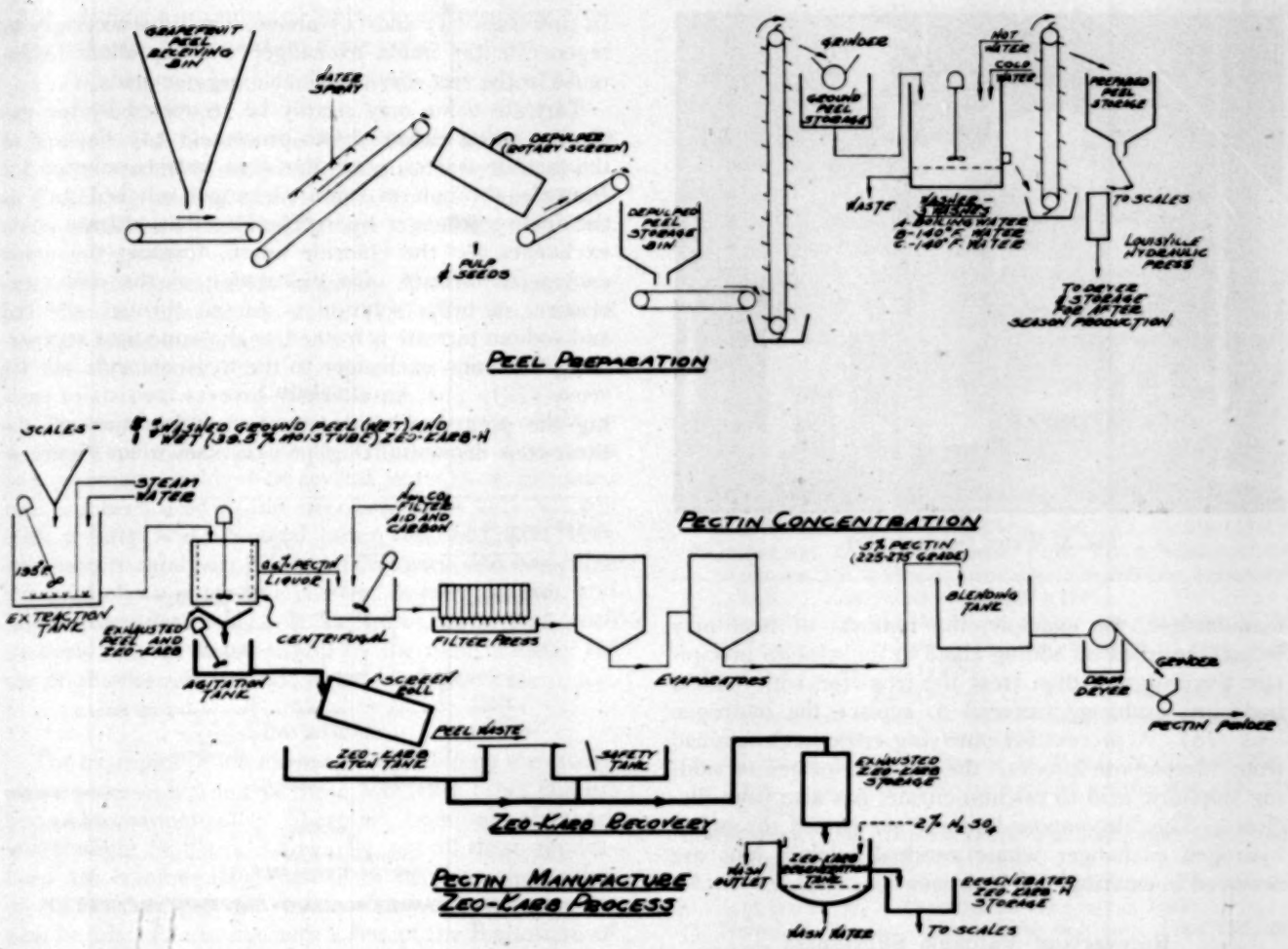


FIG. 2. Diagram of a Hydrogen Exchange Process.

Zeo-Karb exchanges for residual calcium and magnesium in the peel thus lowering the pH and causing the pectin to go into solution. The pectin solution is then separated from the exchanger and peel by centrifuging and the pectin is dried directly without going through the conventional precipitation step with alcohol or aluminum hydroxide. The use of this process results in obtaining a higher yield of a better grade of pectin. The spent peel and exhausted cation exchanger are separated to a certain extent on the porous wall of the centrifuge. The residual peel is separated from the exchanger in a screen roll and catch tank. The ion exchange material is then regenerated with sulphuric acid and rinsed for reuse.

Acid Removal

Acid removal by an anion exchanger is being used on a commercial scale for removing sulphuric acid from beta-alanine solution. B-alanine is used in the manufacture of pantothenic acid. The beta-alanine solution containing 1.2% sulphuric acid is partly neutralized with barium hydroxide. The resulting barium sulphate is separated from the beta-alanine solution by centrifuging and the residual sulphuric acid is removed by passing the solution through an anion exchanger unit. The product is sulphate-free as determined by barium chloride addition. An unexpected benefit of practicing this process is the complete elimination of evaporator foaming which previously was so serious that the constant attention of an operator was required at the evaporator station.

Demineralizing

The combination of the last two types of processes mentioned above, hydrogen exchange and acid removal, in a two-step process, constitutes the demineralizing process (sometimes called de-ionization). This process is being studied on a wide scale in the sugar industry, both beet and cane, for improving sugar yields; in the starch conversion sugar industry for improving yields, improving quality, and plant processes; in the fruit industry for recovering valuable products from waste materials; in polyhydric alcohol manufacture for improving product quality and widening the fields of use for the alcohol. The demineralizing process, as applied to the sugar industry, has been described in the literature in sufficient detail to eliminate the need for discussion here *(6, 13, 15, 17, 4)*. A typical water demineralizer installation is shown in Figure 3.

Addition of Ions

As a corollary to the removal of undesirable impurities by ion exchange, replacing these with more desirable ions, it is also possible to use these processes for the introduction of specific ions to solution. In wine

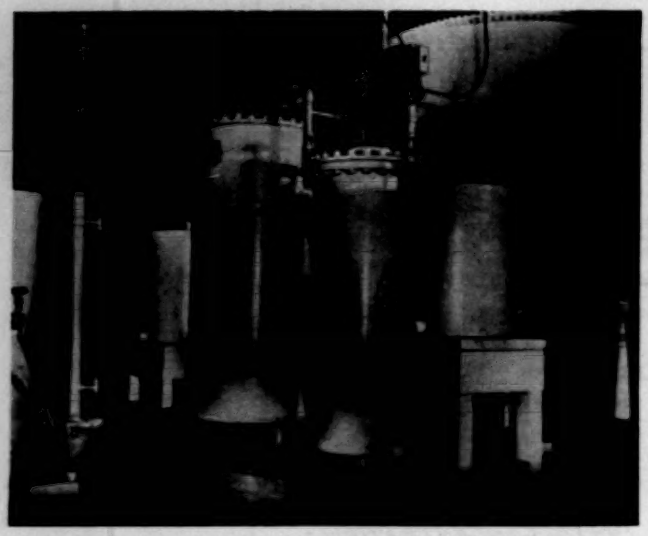


FIG. 3. Water Demineralizer.

manufacture, for example, the removal of iron may be accomplished by adding alkali to the wine to precipitate the iron and then treat the iron-free wine with a hydrogen exchange material to replace the hydrogen ions *(16)*. A process for purifying citric acid obtained from "decompose liquors," the product formed on adding sulphuric acid to calcium citrate, has also been disclosed. The "decompose liquors" are passed through a hydrogen exchanger where residual calcium ions are removed in exchange for hydrogen ions *(3)*.

Recovery of Valuable Substances

Ion exchange has been found extremely useful in recovering valuable substances from solutions. These recovery processes may be brought about by two means:

a. The adsorption of an ionic substance on the ion exchanger and subsequently the recovery of the material from the ion exchanger

b. The recovery may depend on the removal of impurities from the waste solution in order to purify and utilize a non-electrolyte, for example sugar, present in the waste solution.

Valuable acids, such as citric acid, malic acid, aconitic acid, lactic acid, and so on, may readily be recovered by anion exchangers either as the salts or as the acids directly. If the acids are present in the waste solution as salts, they must first be converted to the corresponding acid by passing the solution through a hydrogen exchanger and then through the anion exchanger for recovery. After exhausting the anion exchanger with the acid, it may be treated with

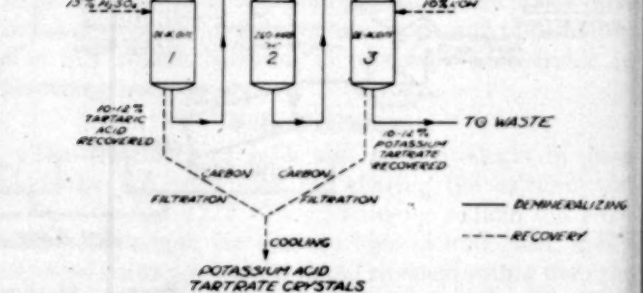
(a) a salt solution, such as sodium chloride, to obtain the salt of the organic acid and the anion exchanger salt

(b) an alkali, to obtain the salt of the organic acid and at the same time regenerate the anion exchanger to the base condition for reuse

(c) an acid, to obtain the organic acid directly, and the anion exchanger salt.

In processes (a) and (c) above, it will be necessary to regenerate the anion exchanger with an alkali before reuse in the recovery of valuable organic acids.

Tartrate value may readily be recovered by ion exchange, using either of two processes: (a) Passage of the tartrate wastes, potassium acid tartrate solution for example, through an anion exchanger salt bed such as the anion exchanger hydrochloride. The tartrate anion exchanges for the chloride anion, forming the anion exchanger tartrate. On exhaustion of the anion exchanger, a brine solution is passed through the bed and sodium tartrate is formed, at the same time regenerating the anion exchanger to the hydrochloride salt for reuse *(12)*. (b) An alternate process consists of passing the potassium acid tartrate solution through the three-step demineralizing process shown in Figure 4.



3-STEP DEMINERALIZING-TARTRATE RECOVERY

The first step consists of an anion exchanger bed, where the free acid in the potassium acid tartrate is removed, forming potassium tartrate solution in the effluent. This is passed through a hydrogen exchanger where the potassium tartrate is converted to tartaric acid which is then removed from solution by the third step, an anion exchanger. The first anion exchanger bed is treated with 15% sulphuric acid to form 8 to 10% tartaric acid solution. The other anion exchanger bed is regenerated with 10% potassium hydroxide to form approximately 10% potassium tartrate. These two solutions are decolorized hot, using activated carbon, mixed, and then cooled. On cooling, large crystals of pure potassium acid tartrates separate *(22)*. It is quite rare that a salt may be recovered in such a convenient manner since there are only a limited number of insoluble acid salts such as potassium acid tartrate.

Alkaloids and other nitrogen compounds such as some vitamins may also be recovered by ion exchange *(10, 8)*. For these processes, cation exchangers are generally used and very often it is necessary to employ liberation and solvent treatment of the cation exchanger in order to effect satisfactory recovery of the nitrogen compounds from the cation exchanger.

Separation of Materials in Solution

A great deal of work has been conducted on the use of ion exchange in the separation of amino acids. Tiselius and his co-workers *(21)* have devised a method

of separating a mixture of amino acids resulting from protein hydrolysis based on these considerations:

1. Only the aromatic amino acids are adsorbed on active charcoal.
2. From the filtrate, the basic amino acids (pI>7) are selectively adsorbed on a carboxylic acid resin.
3. The remaining amino acids (pI<7) are all adsorbed on a cation exchanger containing sulphonic acid groups.
4. Of the amino acids in group 3, only the polycarboxylic monoamino acids ((pI<5) are retained by anion exchangers.

Catalysis

The use of cation exchange in the hydrogen condition as "solid acids" for catalyzing certain types of reactions is a promising field. For several years, a sulphonated coal has been used in the esterification of butyl alcohol with a fatty acid. Several other reactions have been successfully catalyzed in our laboratories. Among these are alcoholysis, ester interchange, de-esterification, and acetal formation *(20)*. It is quite likely that such catalysis may be found useful by the food industry for the production of synthetic flavors and essences.

Limitations

The examples of ion exchange applications mentioned above cover only a few of those which are being studied and used commercially. There are hundreds of others which might be listed. Obviously, not all these applications are commercially feasible in all cases since other means for carrying out reactions are often cheaper. It may be advisable to list here a few of the limitations of ion exchange:

a. Ion exchange has not been succesfully employed as a continuous operation
b. Ion exchange shows up to best advantage in treating solutions having relatively low concentrations of impurities.
c. Dilution of solution being treated or recovered.
d. Amount of regenerant is of necessity greater than theoretical.

Summary

The examples discussed above illustrate how ion exchange has been used in the food industry. The most widespread use is in the removal of impurities but it is also possible to use ion exchange for the addition of specific ions to solutions, the recovery of materials from wastes, the separation of substances in solution, and in catalysis.

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Commercial Ion-Exchange Purification of Sugar-Bearing Solutions[a]

B. N. DICKINSON

Chemical Process Company, Redwood City, California

A discussion of the various developments associated with the application of ion-exchange for purification of sugar-bearing solutions is presented. Typical analytical data pertinent to the discussion are presented.

The use of ion-exchange for the purification of sugar-bearing solutions has received major attention almost from the very moment of inception of ion-exchange principles, especially since the advent of acid-adsorbing resins. In the discussion to follow, I should like to outline in broad terms the growth and development of ion-exchange purification of sugar-bearing solutions with some specific technical data selected to illustrate the various points. Emphasis will be on developments with which Chemical Process Company has been most closely associated, especially those occurring in the Western area.

As early as 1940, Maui Pineapple Company in conjunction with Chemical Process Company began the investigation of the feasibility of salvaging sugar values from pineapple wastes, work which was not completed; but subsequently a similar project was carried on to successful conclusion by Hawaiian Pineapple Company.

Recovery of sugar values from fruit wastes by ion-exchange now seems slated to grow to respectable proportions, especially in the Western areas where water conservation drives are rapidly eliminating water pollution from food-processing and industrial wastes. For example, in the San Jose, California, area, the various packing plants have arranged to dispose of fruit wastes at a central plant. Such wastes assembled without regard for sanitation have but little commercial worth; however, sugar values recoverable by ion-exchange are present. There is a considerable margin over costs of chemical regenerants and plant amortization to pay for collecting and for other process charges. Perhaps the two most difficult technical problems to be solved consist of instituting proper sanitary measures in handling the wastes and of devising proper defecating techniques for the raw juice.

Mr. Henry Vallez, pioneer in the application of ion-exchange to the purification of sugar juices, climaxed years of bench and pilot plant studies with a semi-commercial test during the 1941 sugar beet campaign in the Mount Pleasant, Michigan, plant of Isabella Sugar Company in cooperation with Infilco, Inc. of Chicago. Also in the 1941 campaign Chemical Process Company operated a pilot plant in the purification of sugar beet juice in the Betteravia, California, plant of Union Sugar Company and about the same time The Dorr Company of New York was carrying on experimental work with The Amalgamated Sugar Company at the latter's factory in Burley, Idaho.

In the early part of 1942, Chemical Process Company, working jointly with Corn Products Refining Company, tested in a pilot plant setup the purification of corn starch hydrolysates in the latter's Argo factory—a study which demonstrated the need for an anion exchanger inert to irreversible reaction with dextrose.

Cooperative research involving The Dorr Company, Infilco, The Amalgamated Sugar Company and Chemical Process Company extended ion-exchange studies to include the treatment of raw and predefecated juice. As an outgrowth of this work, Amalgamated built their D. I. plant at Twin Falls, Idaho, in 1946 to handle diffusion juice directly by ion-exchange with the pectin and albuminous material precipitated by the pH drop in the cation bed being filtered out ahead of passage of the acid juice through the anion cell. As a result of delays in installing and the normal engineering difficulties incident upon starting a new plant, a complete evaluation of the process was not made—in fact, the only prolonged run which the plant was able to make extended from some time on Thursday, February 13, 1947, to about 4 p. m. Friday, February 21, a period when white sugar was made commercially without a pound of lime entering the house.

Excessive inversion losses were successfully prevented while retaining the juice in an acid state long enough to carry out the intermediate filtration between anion and cation. For example, a composite sample of raw diffusion juice from one 8-hour shift shows 1.78% invert while a composite sample of evaporator thin juice from the same period shows 1.98% invert, an increase of exactly 0.2%. A number of schemes for handling remelted raw sugars by recirculating through early steps in the process were also employed during the brief run, making it difficult to assess the purity gains. However, an apparent purity of 94.9% in the evaporator thin juice derived from deionization was noted during one period when the apparent purity of the diffusion juice ran in a range from 83.4 to 85.8%. It is not certain that this evaporator thin juice was free of impurities from remelted and recirculated low-purity sugars. The filtration of the acidified juice from the cation cell was carried out successfully, albeit at the expense of considerable filter aid, slightly under 1% on beets. There was no opportunity to determine whether this represented the minimum amounts usable. Precipitated albuminous material in the cells was quite easily removed during the backwashing, but the excessive foaming of the raw juice did create difficulties, which, however, would be easily solvable. Sufficient organic material escaped removal through ion-exchange and filtration to raise the turbidity of the final white sugar from a normal 2 p.p.m. to about 10 p.p.m.

During the period of extreme sugar shortage just past, the processing of beet and cane molasses into an

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unrationed confectioner's syrup by ion-exchange was extensively studied and the practice was adopted commercially in a number of places.

A pilot plant study which attracted considerable attention in the sugar industry was made in 1945 by American Cyanamid Company in cooperation with Holly Sugar Corporation in the latter's Alvarado, California, factory. A report of this study has been given by Riley and Sanborn *(7)*.

The use of exchangers to purify corn sugar liquors has gone forward, meanwhile, in both enzyme and acid hydrolyzed starches with hot processing gaining attention in this field. The Illinois Water Treatment Co. built a plant for purifying maltose at Waverly, Iowa, and Corn Products Refining Company has been making extensive and large-scale pilot plant investigations of ion-exchange production of dextrose in their Argo factory, studies which have led to commercial plants now under construction at Pekin, Illinois, and Corpus Christi, Texas. Increased shelf-life and a better quality of product are the attractive features of ion-exchange to the corn sugar industry.

Second carbonation juice was treated in the Hardin, Montana, plant of Holly Sugar Corporation and the Layton, Utah, plant of Layton Sugar Company, during the 1947-48 campaign, answering on a commercial scale questions regarding increased extraction, regenerant consumption, amount of inversion, dilution, etc. Representative results from these two operations are summarized in Table 1 *(3, 5)*.

TABLE 1

Average Performance of Commercial DI Plants in Purification of Second Carbonation Beet Juice

	Hardin Plant		Layton Plant	
	Influent	Effluent	Influent	Effluent
Brix	12.5	9.6	13.0	11.8
Sugar	11.0%	9.5%	12.1%	11.6%
Purity	89.6%	97.6%	93.8%	98.6%
Non-sugar removal	.....	82.5%	.....	77.6%
Nitrogen-removal	.....	56.0%	.....	.....
Ash-removal, approx.	.....	94.0%	.....	.....
Dilution	.....	13.6%	.....	.....
Invert	.....	.....	0.13%	0.36%

Intermediate syrup from the sugar house rather than second carbonation juice was selected for purification by ion-exchange in the West Jordan factory of the Utah-Idaho Sugar Co. *(1)*. The purified juice was returned to the thin juice going to the evaporator.

Their results are summarized as follows: By handling one-fourth of the machine syrup through a DI plant and returning the purified effluent to the incoming second carbonation juice, some 20% of the impurities entering the house were removed by deionization, and gave an increased sugar yield of 4.04% based on the average extraction for the previous 10-year period, or 4.7% based on the average extraction for the previous 5-year period. The results at Hardin and Layton show a non-sugar removal between 77 and 83%, and it is thought among the more optimistic people in the sugar industry that such a non-sugar removal can be made to lead to an increased extraction of perhaps 11% over a straight house.

Some of the problems encountered in the campaign were rather surprising. Although more white sugar was secured without remelting from the deionized effluent than is possible from standard juice, staining did occur on crystals boiled from intermediate syrups possessing higher purities than the juice which enters the white pan in conventional houses where highly colored juices of 89 to 94% purity give white crystals. Also, despite the 50-60% nitrogen-removal effected by deionization, color did develop rather quickly and intensely upon boiling, an effect apparently catalyzed by iron since it was greatly mitigated when the deionized juice was concentrated in glass. The small quantity of invert formed by deionization may enter into browning reactions with nitrogen-containing molecules to form color bodies of high staining propensity. Normally such invert as enters the house from low-quality beets is destroyed by heavy liming. The cane industry has always had to contend with high invert and colors with pronounced staining tendencies. The suggestion was also made during the campaign that the deionization was removing impurities which normally buffered against color staining of the crystals. A number of solutions to the color problem are indicated as being feasible. More rapid handling of the juice during sugar-boiling would minimize color-formation. It can be inhibited to a certain extent by post-deionization treatment. A more complete nitrogen-removal would further minimize the problem.

Perhaps the most promising of the proposed solutions to the color problem lies in the isolation of nitrogen-containing molecules in the initial stages of deionization. Nees and Bennett *(6)* have indicated a method for isolation and concentration of glutamic acid from Steffens house waste. The waste is run into a cation cell until the nitrogen breakthrough occurs. Then the effluent is collected in a separate nitrogen-rich fraction with the Steffens house waste continuing to enter the top of the cation cell so that its ash content can dislodge the nitrogen which has collected in the bottom zone. When the cell is completely loaded with Steffens house ash, with all the nitrogen pushed out into the nitrogen-rich fraction, then it is rinsed to remove untreated material for recycling, and regenerated. Meantime, the nitrogen-rich fraction is run through a fresh cation cell until nitrogen breakthrough occurs. It is then shut down and the carbohydrates are washed out. The glutamic acid is stripped from the second cell with ammonia in a relatively pure state, some seven or eight times as concentrated as in the original solution. The ash from the nitrogen-rich fraction is left in the second cell.

The Dorr Company in their Westport Laboratory have been studying the distribution of the nitrogen pickup from factory thick juice among the various exchanger beds and regenerant liquors with the results shown in Table 2 *(4)*. Nitrogen-removals of these magnitudes were made with thoroughly regenerated beds. The cation bed is removing well over half the nitrogen content including the valuable glutamic acid and small quantities of glutamine which are convertible to glutamic acid. The anion bed handles among other

TABLE 2

Distribution of Nitrogen Removed by Ion-Exchange from Thick Juice

	1st Factory	2nd Factory
Nitrogen in ammonia used to strip cation bed ahead of regeneration	0.170	0.104
Nitrogen in waste acid cation regenerant	0.004	0.004
Nitrogen in waste anion regenerant	0.089	0.062
Nitrogen in deionized juice	0.010	0.006
	0.273	0.176
Nitrogen in original thick juice treated	0.278	0.178

Units referred to equivalents nitrogen per liter of thick juice.

constitutents pyrrolidone carboxylic acid, also convertible to glutamic acid.

We now consider that it may be desirable to split the cation function in beet sugar refining into three parts: Lime salts which are very inefficiently removed with a sulfuric acid regenerant from ordinary strong-acid-type cation exchangers can be handled by a cation exchanger, such as our Duolite Cation Selector CS-100, which depends for its ion-exchange activity on weak acid groups. This material, which selectively removes the divalent ions over the monovalent ones, and almost quantitatively so long as the pH is buffered against dropping by suitable anions, is efficiently regenerated with dilute acid, perhaps by the waste acid from the standard DI unit. Some of the cation exchangers showing high ash-removal capacity are very poor on picking up molecules containing nitrogen and could thus be utilized in the second phase of the cation exchange for removing the metallic cations, allowing most of the nitrogen to escape by leakage. Finally, a cation exchanger especially adapted to handle large nitrogen-containing cations could complete the exchange and the nitrogen molecules could be stripped in a fairly pure and concentrated solution by the use of ammonia. Acid adsorption may need to be interposed in the process.

Other than some complications in engineering, the chief drawback to the proposal may be a tendency to increase invert. It has been observed that the hydrogen form of a high capacity cation exchanger is an excellent catalyst to promote the inversion of sugar but that impurities in the influent reduce greatly such inversion; hence the universal adoption of the single-pass method of purifying beet sugar juices. Regardless of merits of the above proposal, or modifications of it under consideration, it is desirable to effect as complete a separation of the nitrogen fraction in the initial stages of deionization as possible, both to reduce color problems and to isolate a valuable by-product, glutamic acid.

Sugar cane juice has been subjected to much investigational work in ion-exchange but handicaps in applying this method of purification arise from the high cost of regenerants prevailing in many cane-growing areas as well as the refrigerating costs in cooling juices to prevent inversion. Further, in Cuba the amount of raw sugar which the island is permitted to export to the United States fixes production rather than any limitation of acreage or extraction per ton. Ion-exchange does offer some promise in refining raw cane sugar, however, especially in permitting installations for sugar-refining much smaller than present bone char plants.

Considerable attention has been given to maintaining the capacity of exchanger beds in the treatment of the complex sugar-bearing solutions. It has long been recognized that organic bases are taken up so strongly by the cation exchangers from certain types of solutions that the ordinary acid regeneration is insufficient to dislodge them and it is necessary to resort to occasional caustic rinses to prevent poisoning of the cation bed. It has also been recognized that certain undetermined compounds can be adsorbed and held so tenaciously by the anion bed that they are removed with difficulty by caustic regeneration and therefore frequent acid rinses with mineral acids have been recommended.

In connection with anion poisoning, a recent conversation with Dr. M. Calvin of the University of California concerning some photosynthesis work which he is carrying on with the green algae *Chlorella (2)* revealed information that seemed significant. Dr. Calvin was employing ion-exchangers in the fractionating and isolation of various products formed by the algae. He found that on the Duolite A-3 which he was employing, considerable quantities of triosephosphate, hexosephosphate and phosphoglyceric acid were adsorbed. The first two acids are readily elutriated with ammonia while the phosphoglyceric acid can be removed only with sodium hydroxide. Dr. Calvin tested a number of acids which probably occur in sugar-bearing solutions and found that the phosphoglyceric acid is the only one not removable by ammonia although large excesses of this latter regenerant may be required. Dr. Calvin's observations are suggestive in view of the fact that the anion beds in some of the sugar installations tend to become poisoned when used with ammonia regenerant alone.

It seems to be essential in extending the life of ion-exchangers to maintain the beds in a clean condition. Cation exchangers which have become fouled with nitrogen-containing molecules and have been allowed to remain in such a condition have shown a distinct tendency to lose sulphur while, conversely, poisoned anion beds seem to lose active nitrogen. Is this a bacterial action or is it due to the formation of very stable amides which weaken the primary bonds of attachment?

At Layton a hot water backwash was employed to keep the beds free of waxes and other materials which might clog the exchanger mechanically.

The role of ion-exchange in color, odor and taste refining of sugar-bearing solutions has been an unforeseen but important development.

Mr. E. R. Stadtman has described some experiments which Miss Victoria A. Haas and he have carried out in the Department of Food Technology at the University of California, in which they employed ion-exchange to study the nature of browning reactions. Using an apricot concentrate, they were able to split the color precursors into a cation fraction containing some 81% of the nitrogen, an anion fraction containing 88% of the acids other than the amino acids, and a neutral fraction containing 98% of the sugars. By blending these various components and studying the rate of color-formation, they were able to make some very fundamental deductions regarding the nature of browning reactions. They have demonstrated that the overall browning is

a summation of at least four distinctly different types of reactions involving reactions between nitrogen-containing molecules and sugars, between nitrogen-containing molecules and organic acids, between sugars and organic acids, and reactions involving only organic acids. As often suggested, glucose is an important constituent of the neutral fraction contributing to browning, but the organic acids have roles of varying importance in contributing to the overall effect. When stored separately, the fractions showed but slight tendency towards color-formation.

The general observation can be made that the nitrogen-containing molecules do tend to be removed from sugar-bearing solutions on the cation bed as the work of The Dorr Company and of Haas and Stadtman already quoted above emphasizes, while many of the color bodies tend to be removed on the anion bed; this is especially true with beet sugar.

As an example of the relationship between color-removal and the adsorption of mineral acids, the data from our own laboratory are given in Figure 1. It will be observed that the anion beds are handling the removal of the particular color bodies under study in a striking manner in addition to their more conventional mineral acid adsorption.

A recapitulation shows that the four sources of sugar-bearing solutions—beet, fruit wastes, starch hydrolysates and cane—have been extensively investigated by bench and pilot plant work and commercial development is actively under way in the first three. Bed maintenance problems are being adequately met. An important supplementary function of the ion-exchangers is to remove nitrogen-containing compounds on the cation bed and color bodies on the anion bed, and to carry on taste and odor refining.

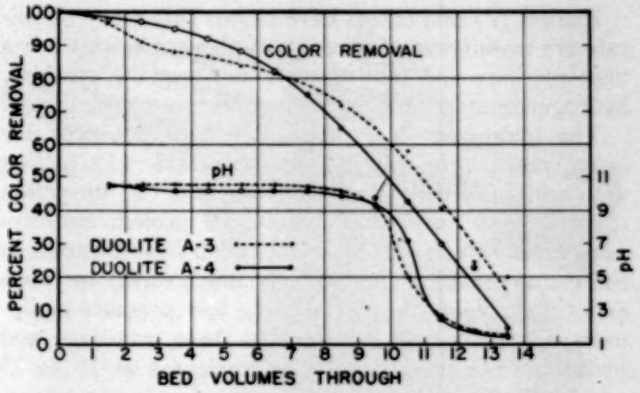

FIG. 1. Simultaneous de-acidifying and decolorizing of "Hydrol" diluted 1:20 and acidified to 0.115 N with HCl. Flow rate = 4 bed volumes per hr.

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Pressure Hydrolysis of Proteins[a]

LLOYD A. HALL
The Griffith Laboratories, Inc., Chicago, Illinois

The possibilities of improving the hydrolysis of proteins with higher pressures and temperatures, such as 75-80 p.s.i. and 180°C., are indicated. A continuous system, whereby hydrolysis might be effected without previous alteration or destruction of the protein molecule, is suggested.

There is considerable literature on the high pressure hydrolysis of various organic substances, but the information is meager concerning the effect of high pressure on proteins. Studies by Sherrard and Blanco *(12, 13)* on the hydrolysis of wood cellulose with water, and, dilute and concentrated acids, have shown that they obtained very nearly the same amount of reducing sugar with 5% HCl at 115 pounds steam pressure in twenty minutes as that obtained by refluxing with 5% HCl in six hours at ordinary pressure. They conducted many studies on the hydrolysis of wood at high pressures and temperatures, and reported vastly accelerated hydrolysis and unobvious reactions.

The cracking of petroleum with high pressure and temperature has produced tremendous volumes of olefine gases most of which have been used for fuel, but which are becoming increasingly valuable as raw materials, particularly for alcohol production. The subsequent effect of these cracking processes on the growth of the petroleum industry is well known and has been given wide publicity.

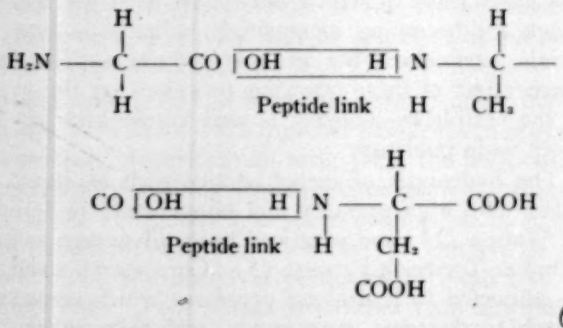
The hydrolysis of carbohydrates such as starch and sugar with high pressure and temperature is described by Walton *(14)* and practiced to an advantage as in the Scholler-Tornesch Process *(5)*. Corn starch itself may be subjected to hydrolytic processes which convert the starch to dextrins, corn syrup, and corn sugar. The hydrolysis is accomplished by acidifying the starch with dilute hydrochloric acid and subjecting the material to steam under pressure.

[a] Presented before the Eighth Annual Food Conference, Philadelphia, Pa., June 9, 1948.

Ipatieff *(7)* and others have shown that many chemicals are manufactured through catalytic reactions using high pressure and temperature, and that the synthetic hydrogenation of some organic substances are facilitated.

The hydrolysis of proteins has been practiced for many years, primarily by three methods (1) boiling with acids, (2) boiling with alkalies, and, (3) the action of proteolytic enzymes. Commercial protein hydrolysates as shown by Hall *(6)* have been manufactured in this country since about 1935, and much earlier in Japan and China. In more recent years, low pressure equipment for protein hydrolysis has been available and hydrolytic reactions have been conducted at 10 to 25 pounds pressure at temperatures ranging from an average of 108° C. to 125° C. Reaction time has therefore been decreased from about 14 to 16 hours to from 5 to 6 hours when the hydrolyzing acid is 20 to 25 percent HCl. This has been important from a profitable production standpoint, but little has been learned regarding the flavor value of the resulting hydrolysate, the liberation of certain amino acids, and, the alteration or destruction of the amino acids.

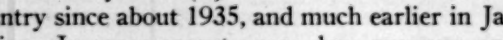
Protein molecules consist of hundreds or even thousands of amino acids. While an examination of the nature of the hydrolysis products has contributed much to our present knowledge regarding the structure and chemical composition of the protein molecule, there remains much work to be done. Investigations from the work of Kossel, Fischer, Abderhalden, Osborne, Svedberg, Bergmann and others have given results leading to the conclusion that the proteins consist of amino acids linked together by means of the carboxylic group of one and the amino group of the other with a simultaneous loss of water, and, further, that secondary valence forces have an important role in the formation of characteristic patterns which are associated with the various proteins. The early postulate of Emil Fischer *(2)* that proteins consist of long chains of amino acids bound through a peptide or amide linkage is still generally accepted. This theory is acceptable on the observation that proteins contain very few free amino and carboxyl groups in contrast with the large number of groups of both types present in the hydrolysate mixture and since, on hydrolysis, amino and carboxyl groups are liberated in equivalent amounts. The manner in which the amino acids are linked to form peptide chains may be indicated in accordance with Example I.



(8)

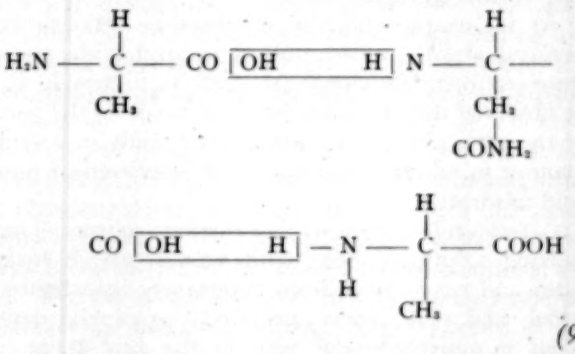
On hydrolytic cleavage, the peptide linkages present in the protein molecule are broken and the constituent amino acids are liberated. In this process, the original protein unit is broken down to proteoses, peptones, and peptides, into the amino acids.

In Example II it is to be noted that ammonia, another compound found in protein hydrolysates, is formed mainly by the hydrolysis of the acid amide group:



The quantity of ammonia parallels the amount of dicarboxylic acids.

In accordance with Example III the carboxyl group of dicarboxylic amino acids not involved in the formation of peptide linkages is found principally in the acid amide form. Although a small amount of ammonia may be produced by the action of acids on the monoamino acids, by far the largest amount is due to the hydrolysis of the acid amide group. The existence of acid amide groups in chains may be indicated as follows:



(9)

It has been demonstrated by Gordon and coworkers, and by Lugg *(3, 10)* that there is an alteration of the amino acid residues during hydrolysis and in certain instances there is destruction of some of the amino acids. The effects of this alteration and decomposition or destruction still remain a problem for further research study in the proper evaluation of hydrolytic reactions.

In studies on the production of hydrolysates, our interest in the effect of high presure and high temperature was primarily from the standpoint of substantially accelerating the hydrolysis time and thus improving the production economics. Predicating our work upon substantial information and data from many sources where such reactions had been successful experimentally and industrially, we realized the potentialities of hydrolysate production of an improved character, and also the possibility of less troublesome extraction of glutamic acid and other amino acids.

It is not our purpose here to discuss in detail the theoretical chemistry of the hydrolysis of proteins by high pressures. We are concerned mainly with presenting the data of some two dozen or more hydrolytic experiments carried out under varying conditions which are by no means complete.

It is well known the higher the temperature, the faster is the rate of hydrolysis. The most frequently used procedure of hydrolysis has been to boil and reflux the mixture containing the catalytic agent and the protein until the reaction is completed. It is known that the rate of hydrolysis can be increased if the mixture is heated under presure. Since time is a factor, it is preferable not to continue hydrolysis beyond the time

required for its completion. The hydrolysis of proteins with acids, and, under certain conditions, with alkalies, under steam pressure has definite advantages over hydrolysis by boiling and refluxing.

Studying the effect of temperature on the rate of acid hydrolysis of gelatin, Greenberg and Burk *(4)* demonstrated that the velocity constant is a function of temperature and can be expressed by the following equation:

$$\text{Log } K_a = 0.0287\,T - 5.30$$

in which K_a refers to the velocity constant at unit activity and T is expressed in degrees centigrade. Obviously there is considerable economy in time and material when proteins are hydrolyzed by acids or alkalies under steam pressure and at an elevated temperature, not sufficiently high to alter the protein prior to hydrolysis.

Dunn *(1)* has investigated the rate of hydrolysis of casein by acids and on the basis of his measurements reported that it was a first-order reaction. Greenberg and Burk *(4)* studied the hydrolysis of gelatin, gliadin, and silk fibroin by different concentrations of hydrochloric acid and sulphuric acid. Their data indicated that the rate of hydrolysis of these proteins measured by the increase of amino nitrogen was that of a second-order reaction. They also showed that the catalytic action of acids in protein hydrolysis is proportional to the activity of the hydrogen ion. Nassett and Greenberg *(11)* reinvestigated the acid hydrolysis of casein and found that its rate conformed to the equation for a second-order reaction:

$$K = \frac{1}{t} \times \frac{x}{100 - x} \times \frac{1}{100}$$

in which x represents the percentage of protein hydrolyzed in time t.

It is well known that the hydrolysis of a protein is considered complete when the peptide linkages are entirely broken. This is rather difficult to determine accurately. The Biuret test is useful and frequently employed for this purpose, but it is not conclusive. One of the best methods for determining the completion of protein hydrolysis is the estimation of the amino nitrogen by means of the Manometric Van Slyke apparatus, and this has been used in our work.

Experimental

Figure 1 shows the high pressure equipment (A Pressure Bomb and Shaker) used in our experiments. The equipment is widely used for synthetic hydrogenation of organic substances and for facilitating the investigation of high pressure catalytic reactions. It is so constructed that the bomb in which the reaction is taking place can be heated, and at the same time the mixture within can be agitated. The bomb is rocked through an angle of approximately 30° at a rate of 58 times per minute which is obtained by the use of a one-quarter horsepower induction motor with built-in reduction gears. There is a pressure gauge mounted between the inlet valves and the pump so that reaction pressure may be determined at all times. The flask or liner used inside the bomb is made of heavy pyrex glass especially built to withstand high pressures and temperatures.

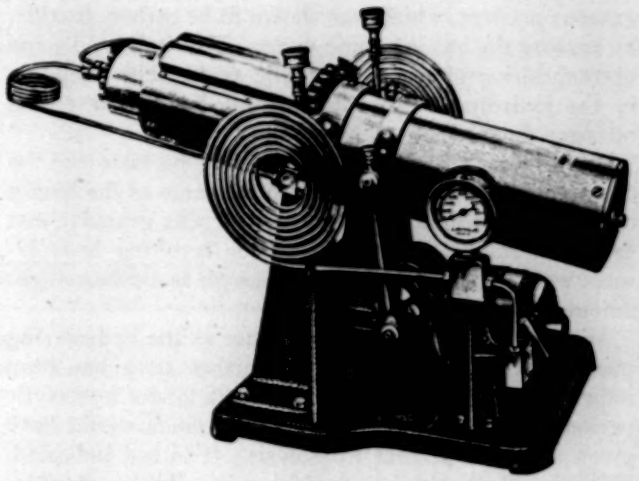


FIG. 1. Superpressure equipment for pressure hydrolysis of organic substances. For pressures up to 22,000 lb. per sq. in. For temperatures up to 500° Centigrade.

Wheat gluten, the only protein used in our studies, contained approximately 85% protein and was a light colored material of about 40 mesh fineness. In all tests, ¼ lb. of gluten was mixed into a paste with 350 ml. of liquid.

Table 1 gives the results of tests with water as the hydrolyzing medium. After the gluten was mixed into a paste it was placed into the pyrex glass tube and heated in the bomb to the desired temperature which was maintained for 30 minutes. After cooling for approximately three hours to room temperature, the bomb was opened and the material removed from the pyrex flask with 50 ml. of wash water which was added to the hydrolysate. The composite was filtered and analyzed in accordance with data given in Table 1. It was observed that the humin residues in tests 1, 2 and 3 were tar-like, resinous and difficult to filter. In tests 1, 2, and 3 the hydrolysate was very dark in color, had a strong scorched odor and bitter taste. The decolorization of these hydrolysates was very difficult. The total period of heating in all of the tests of Table 1 varied from 2½ hours in test number 1 to 5 hours in test number 5. These samples gave off a considerable amount of a

TABLE 1

Hydrolysis With Water as Hydrolyzing Medium

Test	Medium	Time Hrs.	P. s. i.	Temp. ° C.	Sp. G.	Amino N/ Lb. Gluten	Approx. % Hydrolysis	pH	% Humin	Character of Humin
No. 1	Water	.5	225	190	1.0298	3.46	8.65	6.29	6.90	Tar-like
No. 2	Water	.5	310	204	1.0517	10.68	26.70	7.63	6.70	Resinous
No. 3	Water	.5	500	220	1.057	13.07	32.70	8.40	10.23	Tar-like
No. 4	Water	.5	800	225	1.0499	14.80	37.00	8.70	1.80	Granular
No. 5	Water	.5	1000	235	1.0514	17.56	43.90	8.88	1.60	Granular

gaseous product, which was shown to be carbon dioxide, by passing the gas into lime water. This indicated some decomposition of the dicarboxylic acids. The ammonia in the hydrolysate was distilled off before the amino nitrogen determinations were made.

Tests 4 and 5 had the usual hydrolysate taste and the character of the humin was much the same as the humin obtained by ordinary hydrolysis, and was granular and easily filterable. The pH of all the resulting hydrolysates were alkaline, apparently enough to aid in a slight amount of alkaline hydrolysis.

While the experiments with water as the hydrolyzing medium were not carried any further than has been indicated, there is the possibility that a longer hydrolytic period at a presure of 800 to 1000 pounds would have given a higher percent hydrolysis. It is not indicated, however, that complete hydrolysis could be obtained with water alone, or that the amino acids would have been undamaged.

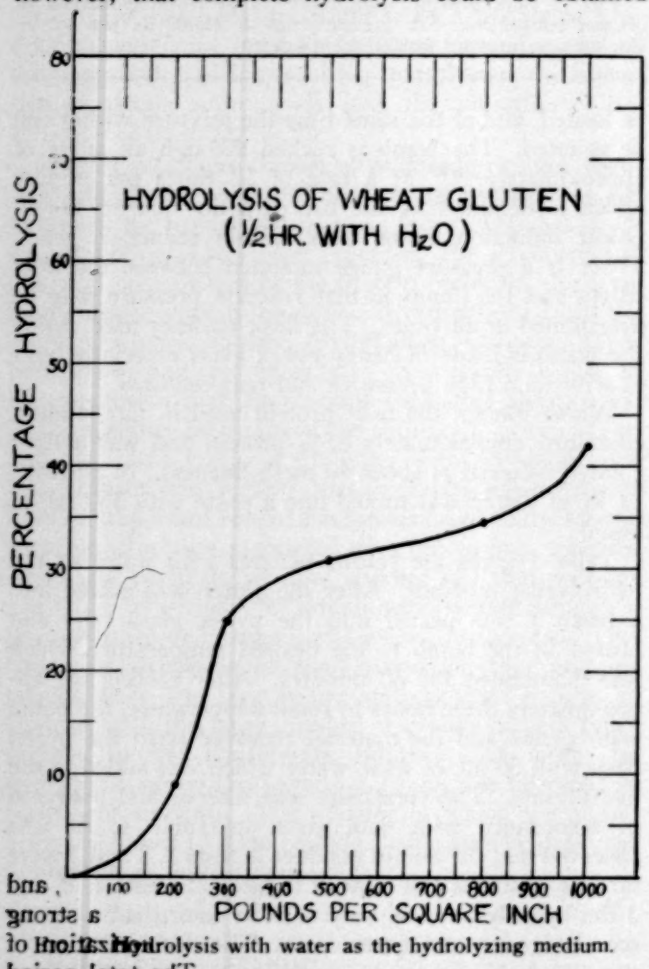


Fig. 2. Hydrolysis with water as the hydrolyzing medium.

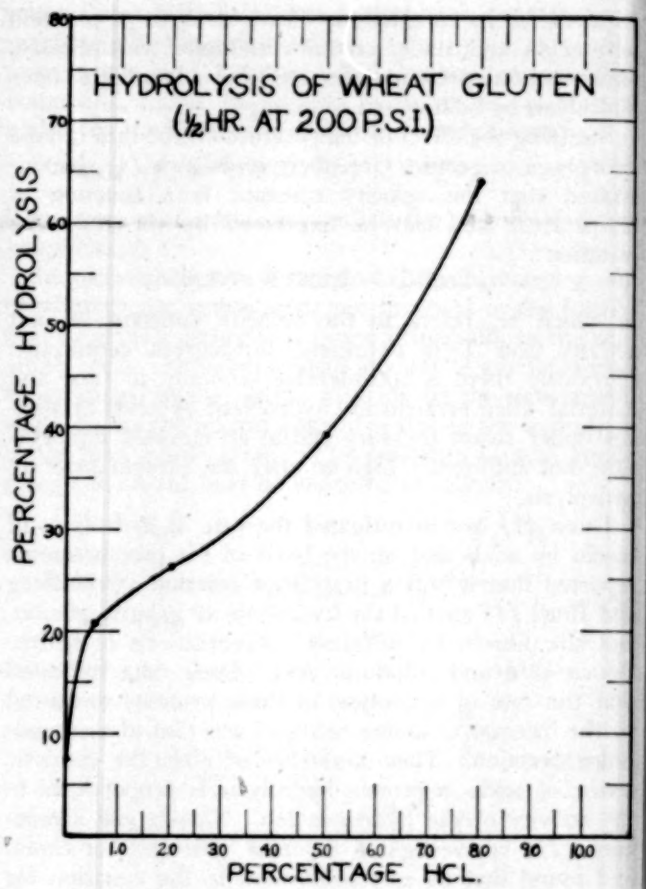


Fig. 3. Hydrolysis in different percentages of hydrochloric acid.

In Figure 2 we have expressed the results of Table 1 by plotting the percentage of hydrolysis against pressure.

Table 2 represents tests using different concentrations of HCl with varying pressures and temperatures. The addition of 1% acid lowered the pH nearer to the neutral point, and probably minimized the effect of alkaline hydrolysis. Results show that less amino nitrogen was formed with acid than was formed without acid, and that a small amount of acid of the order of 0.5% to 5% is of little value in substantially increasing hydrolysis. It is well known that HCl actually combines with some of the amino acids to form the hydrochlorides. We believe the HCl is chemically bound in this manner, and thus not able to act further as a catalyst. Because test 15 with 8% HCl gave an approximate hydrolysis

TABLE 2

Hydrolysis Under Varying Conditions of Acidity, Pressure, and Temperature

Test	Medium	Time Hrs.	P. s. i.	Temp. ° C.	Sp. G.	Amino N/ Lb. Gluten	Approx. % Hydrolysis	pH	% Humin	Character of Humin
No. 6	.5% HCl	0.5	200	210	1.0455	8.8	22.00	.....	8.31	Granular
No. 7	1.0% HCl	0.5	1000	246	1.0485	14.6	36.50	.....	13.00	Granular
No. 8	1.0% HCl	2.0	950	254	1.055	12.44	31.10	.....	9.22	Tarry
No. 9	2.0% HCl	0.5	200	206	1.0522	10.59	26.50	3.88	13.67	Granular
No. 10	2.0% HCl	2.5	925	213	1.054	10.00	25.00	.....	21.00	Granular
No. 11	2.0% HCl	4.0	200	206	1.053	9.14	22.80	.....	10.00	Granular
No. 12	2.3% HCl	1.0	950	251	1.048	9.98	25.00	.....	11.09	Unreacted Protein
No. 13	5.0% HCl	0.5	200	208	1.0569	15.85	39.60	2.45	13.64	Granular
No. 14	5.0% HCl	2.0	200	208	1.0585	14.57	36.43	.....	10.00	Granular
No. 15	8.0% HCl	0.5	200	208	1.074	25.39	63.48	.....	15.40	Granular

TABLE 3

Hydrolysis in 10 Percent Hydrochloric Acid

Test	Medium	Time Hrs.	P. s. i.	Temp. ° C.	Sp. G.	Amino N/ Lb. Gluten	Approx. % Hydrolysis	% Humin	Character of Humin
No. 17	10% HCl	0 *	50 Max.	139	1.093	29.94	74.85	13.70	Granular
No. 18	10% HCl	0 *	120 Max.	172	1.086	29.63	74.08	14.60	Granular
No. 19	10% HCl	0.5	1020	252	1.058	25.57	63.94	13.00	Tarry
No. 20	10% HCl	1.0	825	239	1.068	26.55	66.38	15.00	Granular
No. 21	10% HCl	2.0	125	154	1.086	27.80	69.35	13.30	Granular
No. 22	10% HCl	2.0	500	230	1.073	26.31	65.77	14.00	Granular
No. 23	10% HCl	3.5	125	154	1.087	28.60	71.51	14.60	Granular
No. 24	10% HCl	5.0	100	146	1.086	29.70	74.24	10.60	Granular

* The figure 0 means that the hydrolysate was not held at the maximum, but was immediately allowed to cool.

TABLE 4

Hydrolysis in Weak Solutions of Sodium Hydroxide

Test	Medium	Time Hrs.	P. s. i.	Temp. ° C.	Sp. G.	Amino N/ Lb. Gluten	Approx. % Hydrolysis	% Humin	Character of Humin
No. 25	1.0% H_2SO_4	2.0	200	206	1.056	8.0	19.91	6.28	Tarry
No. 26	0.5% NaOH	0.5	200	206	1.056	10.59	26.47	0.46	Unreacted Prot.
No. 27	1.0% NaOH	0.5	200	206	1.063	10.17	25.40	1.41	Granular-Brown
No. 28	2.0% NaOH	0.5	200	198	1.073	9.91	24.80	4.47	Light Brown

of 63.50%, we attempted to isolate glutamic acid from this sample, according to normal procedures, in an effort to determine whether this constituent could be more readily separated. There was no free HCl present in the vacuum distillate from the hydrolysate filtrate and when the filtrate was adjusted to pH 3.2 there was a heavy precipitate which on analysis was found to be sodium chloride and glutamic acid. This was indicative that glutamic acid could be isolated from such a hydrolysate providing the degree of hydrolysis was sufficiently high.

In Figure 3 is shown graphically the approximate hydrolysis from results given in Table 2.

Increased amounts of HCl were used at varying pressures and temperatures, and while it was noted that the maximum amino nitrogen is much lower than the expected theoretical value, it is obvious that the increased acid assists tremendously in the hydrolysis of the protein. The appearance, smell and taste of the various test samples indicated that there had been some destruction of the protein prior to hydrolysis, particularly in tests 19 and 20 where very high pressures and high temperatures prevailed. In test 20 the hydrolysate was further hydrolyzed with acid, representing a total acid concentration of 14% HCl to determine if the nitrogen which was unaccounted for could be released as amino nitrogen. An additional six-hour hydrolysis by refluxing at 113° C. gave an increase in hydrolysis from 66.38% to 88.37%.

Regular procedures for the separation of glutamic acid were used and a yield representing 40% of the expected glutamic acid was obtained. It is apparent that the protein had been altered in some manner, prior to hydrolysis, and that the glutamic acid separation had been impaired. This again indicates that protein damage occurs at high pressures and temperatures. It was interesting to find that excellent hydrolysis was obtained with 10% HCl as in tests 17 and 18, but it is strongly apparent that sufficient acid must be present to form the acid salts of all of the amino acids present in the protein before complete or nearly complete hydrolysis can be obtained.

Thus far, these studies have indicated that temperature and pressure together, or pressure alone, will not hydrolyze proteins unless the acid requirement is met. High temperatures actually destroy, or partially destroy the protein molecule, rather than hydrolyze it.

In tests 17 and 18 the hydrolytic reaction occurred at fairly low pressures, but much higher than have been commercially considered. It is interesting to observe that the approximate degree of hydrolysis is relatively high, and yet the acid is no stronger than in the other tests. This would seem to indicate that more efficient hydrolysis can be obtained at a lower pressure.

In attempting to obtain a more complete picture concerning the effects of high pressures of proteins with different catalysts, test No. 25 with 1% sulphuric acid was made. The results were not sufficiently encouraging to continue work with this acid, and, in our opinion, were not comparable with the same strength HCl. It was not thought desirable to go to higher pressures than 200 lbs. per square inch because of previous results which indicated protein destruction.

Also in Table 4 we have shown the results obtained in three tests with weak solutions of sodium hydroxide. These were not satisfactory even though the amount of hydrolysis compared favorably with that obtained with comparable amounts of HCl. It is known that sodium hydroxide destroys some of the amino acids and racemizes certain others. Also noted in these three tests was the unusual amount of ammonia liberated.

TABLE 5

Rate of Hydrolysis Under Usual Commercial Conditions

Test	Medium	Time Hrs.	Temp. ° C.	Amino N/ Lb. Gluten	Approx. %* Hydrolysis
No. 1	20% HCl	8	108-110	35.8	89.50
No. 2	20% HCl	9	108-110	38.5	96.25
No. 3	20% HCl	11	108-110	39.8	99.50

* Complete hydrolysis of wheat gluten (85% protein) gives 40 grams Amino N/lb. gluten.

In Table 5 data is given to show the effect of time upon the percent of hydrolysis of the same gluten at the ordinary temperature and pressure used for the commercial production of hydrolysates with 20% HCl. Almost complete hydrolysis can be obtained in eleven hours at temperatures of 108-110° C.

Discussion

The odor and appearance of the hydrolysate in tests 17 and 18 in Table 3 were very comparable to hydrolysates obtained by current hydrolysis practices, and it is thus logical to assume that the protein has not been adversely affected or destroyed prior to hydrolysis by this treatment. It is our concept that some pressure between 50 and 100 pounds is indicated as optimum where maximum hydrolysis can be obtained without protein destruction. For these pressures to be practical commercially, the hydrolysis time would necessarily have to be considerably less than by the present reflux treatment.

Further preliminary tests have indicated 75 to 80 pounds pressure is about the maximum that can be used without destruction of protein, and it has also been indicated that the maximum acid requirement for such a procedure would be from 16 to 17% HCl. We have determined that complete hydrolysis can be obtained by refluxing the protein with 18% to 19% HCl in a period of 15 hours. Therefore, it is reasonable to suppose it would be practical to hydrolyze under a pressure of 75 lbs. with 16% HCl and that complete hydrolysis could thus be obtained.

There is the real possibility that the hydrolysis of wheat gluten and related protein materials could be hydrolyzed by making a slurry of the protein in an acid of appropriate concentration, and that this slurry could be passed through a heated tube to bring about hydrolysis. Perhaps the slurry could be forced through by gravity or perhaps some type of screw mechanism could be devised to keep the slurry moving at a predetermined speed through the heated pressure tube. The advantage of the flow method over the static method is that very little time would be required for heating and cooling. Hence the time of contact in the hot zone would be under much better control. Probably such a continuous system could be devised which would, to some extent, revolutionize the production of protein hydrolysates.[b]

Summary

We have indicated the possibilities of improving the hydrolytic reactions of proteins with high pressures and temperatures, and it is our belief that several opportunities exist for improving the production of protein hydrolysates with moderate amounts of pressure, such as 75 to 80 pounds per square inch, and at temperatures up to 180° C. by a continuous system in a relatively short time whereby hydrolysis can be effected without previous alteration or destruction of the protein substances.

Since the experiments which I have described could have been much more exhaustive, it is clearly evident more work can be done on this problem, particularly from the theoretical standpoint, to uncover valuable information. I believe there are definite indications these more thorough fundamental studies could change our present concepts and knowledge concerning protein hydrolytic processes and be further translated into improved industrial procedures and products.

[b] Unpublished information has been presented to the author indicating that a continuous system for the hydrolysis of proteins is in commercial operation.

Acknowledgment

The author acknowledges the assistance of Dr. Charles D. Hurd and Mr. George Bernard of Northwestern University, for the use and supervision of the pressure equipment and tests made with same, and, to Mr. Robert M. Stephenson[c] and other associates of the Griffith Laboratories for analytical data.

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[c] Now with Basic Vegetable Products Co., Vacaville, California.

The Influence of Heat-Processing on the Functional and Nutritive Properties of Protein[a]

DANIEL MELNICK[b] AND BERNARD L. OSER[c]

The concept of food protein as N x 6.25 should be extended to include a consideration of both the functional and nutritive properties of the protein. The functional properties may be evaluated by the use of standardized procedures for determining the degree of protein denaturation; the nutritive properties, by determining the amino acid composition of the protein and its susceptibility to enzymic digestion. For optimal utilization of food proteins all essential amino acids must not only be available for absorption but must also be liberated during digestion in vivo at rates permitting effective mutual supplementation.

Since the major portion of this report deals with the susceptibility of protein to enzymic digestion as a factor in nutrition, the title of this paper might have been "The Influence of Heat-Processing of Protein on the Availability of the Amino Acids" in keeping with the subject of this symposium. However, the functional properties of protein, also readily modified by heat-processing, affect appearance and palatability of the end-product and therefore cannot be dissociated from the nutritive properties of the protein. Food must be consumed in order to make its nutrient contribution.

It is not uncommon for a given food material to be processed in different ways without a change in its protein content or in the amino acid composition of the protein complex; yet these foods cannot be used interchangeably without modifying the quality of the end-product. Many food technologists, aware of the limitations of their raw materials, usually strive to attain food formulations which give optimal performance, the functional properties of the protein receiving primary, if not exclusive, consideration. On the other hand, the nutritionist concerns himself with the influence of processing on the nutritive value of the protein, striving to avoid destruction of the essential amino acids and to attain a product free from factors which might interfere with the availability of the amino acids. Ease of manufacture, appearance, palatability, stability, and ready utilization of the end-product, however, are important considerations and cannot be sacrificed even for optimal nutritive value. The ultimate aim, of course, should be to obtain a food or a blend of food materials in which both functional and nutritive properties of the protein are at a maximum. However, this can be realized only in a few instances.

The experience of manufacturers of soy products will serve as an excellent illustration of the problem to be presented. Soy flour is used principally as (a) a wheat flour extender, (b) as a source of "tofu," an oriental main dish prepared by forming a curd from the water-extractable soybean protein, and (c) as a major ingredient in low-cost food preparations such as soup mixes. For the first two purposes it is essential that the protein in the product be in an undenatured form so that the functional (utility) properties will be unimpaired. However, in those food products consumed after minimal heating at atmospheric pressure and in which the functional properties of the protein are not critical, the soy product should be adequately processed to increase the biological value of the protein to a maximum. In raw or insufficiently heat-processed soy products an anti-tryptic factor is present *(8, 28)* which interferes with the digestibility of the soy protein; excessive heat-processing effects a reduction in the nutritive value of the protein *(79, 62, 10, 20, 25)*. The soy industry has been aware of this situation and has attempted to manufacture products under standardized conditions to serve specific functions. A soy flour intended for use in bread may not be satisfactory for inclusion in a soup mix and vice versa. The group at the Quartermaster Food and Container Institute has prepared a specification for the procurement of soy products which unfortunately fails to recognize these differences. Simple objective test methods are not available at the present time for inclusion in a specification to justify setting specific requirements for the two kinds of soy flour desired; namely, for baking bread and curd formation, or for soups, nährmittel, etc. A collaborative research project with the soy industry is now under way for the purpose of incorporating in the specifications suitable methods of test to insure that flour required for a specific purpose is procured. Too frequently have reports been received from overseas areas severely criticising the soy products furnished. Heat-processed soy meal containing practically no water-extractable protein has been furnished to the civilian populace in the Orient, whereas low heat-processed soy flours have been shipped to Germany for inclusion in soup mixes and food combinations which are consumed either as such or after minimal heating.

The Functional Properties of Protein in Food Materials

In Table 1 are shown five food products subjected to heat-processing with the view toward obtaining products whose protein components exhibit desirable functional

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[a] This paper from the Quartermaster Food and Container Institute for the Armed Forces, as one of the participating laboratories, has been assigned Number 189 in the series of papers approved for publication. The paper was presented by the senior author in a symposium on "Amino Acids" before the Institute of Food Technologists, Eighth Annual Convention, Philadelphia, Pa., June 9, 1948. The views or conclusions contained in this report are those of the authors. They are not to be construed as necesssarily reflecting the views or indorsement of the Department of the Army.

[b] Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill. The experimental work on the nutritive properties of proteins was carried out while the senior author was associated with Food Research Laboratories, Inc., Long Island City, N. Y. Certain phases of this work have been reported elsewhere *(60b, 52, 61)*. Studies on both the functional and nutritive properties of proteins are continuing at the Quartermaster Food and Container Institute for the Armed Forces.

[c] Food Research Laboratories, Inc., Long Island City, N. Y.

TABLE 1

Influence of Heat Processing on the Functional Properties of Protein

Protein Source	Heat Processing	Resulting Product
Soybean	100°-135° C. for 10-0.1 min.	Slightly denatured protein (50-65% extractable) enzyme (diastase and lipoxidase) inactivated; used for curd formation in main dish preparations, in bread manufacture, in sausages, and in alimentary pastes.
Egg	Preheated to 60° C., spray drying to 2.0% moisture content, temperature <65° C.	Slightly denatured protein; used to form a coagulum in main dish preparations, for imparting proper body and structure to baked products, spaghetti, etc., for emulsifying properties.
Yeast	28-30° C. to 8% moisture content.	Enzymes in intact cells still active; used as a fermentation agent in baking industry.
Flour	55-80° C. for approx. 1 min.	Accelerated maturation, replacing agene process, with the result that concentration of free sulfhydril compounds is reduced without impairment of the diastatic activity; used in baking industry.
Milk	90-92° C., 15 min.	Denatured protein, enzymes inactivated, low in volatile sulfhydril groups; used in bread manufacture.
	70-72° C., 15 sec.	Slightly denatured protein, which redissolves in preparing beverage.
	61-62° C., 30 min.	Slightly denatured protein in frozen pasteurized milk.

properties. Additional materials could have been included, but those listed will suffice for this discussion. Excessive heat-processing (or minimal heating in the case of the milk powder employed for baking purposes) alters the character of the products to such an extent that they can no longer be employed for the purposes intended.

Rather than depend solely upon subjective utility tests for rating food materials in which the functional properties of the protein are susceptible to change during processing, it is suggested that precise, objective tests of the extent of protein denaturation be employed. Of course, in each case correlation between the utility and biochemical tests has to be established. Various laboratory procedures for ascertaining the degree of protein denaturation are already being employed in the laboratories of the food industries. Thus in the case of soy and egg protein, measurements of the solubility index are usually made. The results of these tests characterize the functional properties of the product at the time the material is freshly prepared; these, however, do not reflect the potentialities of the protein to undergo further changes during the subsequent storage periods, with a resulting decrease in solubility. It would seem desirable to supplement the solubility measurement with tests to ascertain whether or not the salting-out characteristics of the protein have been altered. Denaturation of coagulable protein can occur without precipitation *(51)*. Since the salting-out characteristics of proteins change under conditions inducing incipient denaturation, a quantitative salting-out test should serve as a better indicator of the actual degree of denaturation effected, than the simple solubility measurement, and of the tendency of the protein to become insoluble during prolonged storage. Harland and Ashworth *(29)* have used the salting-out behavior of the whey protein in dried milk for purposes of classifying dried skim milk samples with respect to baking qualities. In this case protein denaturation is desired. At the Institute a precise objective protein precipitation technique has been evolved for rating the potentialities of soy preparations for curd formation. This same procedure may prove to be useful in rating soy flours for use in bread manufacture.

Influence of Heat-Processing on the Nutritive Value of the Protein

The above title constitutes the major theme of this discussion. Three factors, broadly speaking, determine the nutritive value of a food as a source of protein. These are (a) the quantity of protein expressed in terms of total solids content, (b) the essential amino acid composition of the protein, and (c) the availability of the amino acids for tissue protein synthesis.

It is incorrect to compare two food products on the basis of their protein content alone without reference to their total solids content. Reference is sometimes made to the protein value of a cereal or leguminous food relative to that of meat. In terms of total solids, materials like oats or soybeans furnish much less protein than meat, even though their net protein content may be the same or even greater.

The chief consideration in the selection of dietary proteins is their content of indispensable amino acids, since proteins can be no better than the biological sum of their amino acids. Animal proteins have greater biological value than vegetable proteins because they contain a greater number of essential amino acids in quantities of nutritional significance. The essential amino acids, however, must not only be present but must be readily available for absorption and utilization by the animal organism.

Papers have appeared demonstrating with a number of species that the protein in properly heat-processed soy products has a much higher biological value than that of the raw bean *(60, 55, 34, 35, 79, 33, 62, 32, 2, 15, 54, 20, 25, 46, 77)*. Supplementation with methionine eliminates in large part the discrepancy between the two products *(32, 2, 54, 20, 11, 68)*. In addition to soybeans, many other leguminous proteins seem to improve in nutritive value after the application of heat *(38, 75, 22, 76, 23, 21, 67)*. On the other hand, excessive heating has been shown to impair the biological value of proteins. The adverse effects of heat on milk proteins (particularly casein) *(56, 27)*, on edestin *(73)*, on blood globin *(14)*, and on the proteins in cereals *(56, 44, 58, 71, 43, 64)*, meat *(57, 69, 30)*, and fish products *(50, 66, 78)*, in soybean *(79, 62, 10, 20, 25)*, and other legumes *(83, 21, 59, 67)*, in coconut

meal *(54)*, and in a baking formulation *(6)* have been reported. In practically every case supplementation of the ration with lysine effected substantial if not complete correction of the nutritional deficiency.

Storage of foods over extended periods of time appears to cause impairment in the nutritive value of the protein similar to that noted following excessive heat-processing. This has been observed in studies with dried skim milk *(36)*, with soy meal *(41)*, and with the cereal grains *(41, 42, 40)*.

Throughout the papers indicated above, data are presented demonstrating that heat-processing of foods has a profound effect on the nutritive value of the protein without affecting the protein content, the essential amino acid composition, or the degree of protein digestibility *in vivo*. Despite the fact that methionine is the principal limiting amino acid in raw foods, whose protein value can be improved by heat-processing, and that lysine plays a corresponding role in the case of excessively heated proteins, the contents of these amino acids are not materially affected by the processing techniques. This had led to the conclusion that these amino acids in the ineffective proteins are not biologically available *(84, 17, 7)*. In the case of lysine it has been postulated *(17, 7)* that a new peptide linkage forms as a result of heat-processing involving the ϵ-amino group of lysine and the free carboxyl group of a dicarboxylic amino acid and that this complex is resistant to enzymic digestion.

In the present report data will be presented demonstrating with heat-processed proteins that the *rate* of enzymic liberation of the amino acids, rather than *degree* of amino acid availability, is of critical importance in determining the efficiency with which the absorbed amino acids are retained for tissue protein synthesis. In studies of amino acid supplementation of deficient amino acid mixtures *(18, 26, 9)* or of an inadequate ration *(4)*, or of proteins supplementing each other *(37)*, time intervals have proved to be exceedingly important; as the time interval between feeding the dietary components is reduced more effective biological responses are obtained. As a working hypothesis in the present studies, it has been postulated that for optimal utilization of protein all essential amino acids must not only be available for absorption but must be liberated during digestion *in vivo* at rates permitting mutual supplementation, and that heat-processing influences the relative rate of liberation of the amino acids. That amino acids are liberated during digestion at different rates has previously been reported *(53)*, but the nutritional implications of such observations have been largely neglected. Other factors, such as the presence in foods of naturally occurring toxic agents *(45)*, vitamin-amino acid interrelationships *(53b)*, or the necessity for the animal organism to absorb amino acids in certain peptide linkages *(80, 81, 82)* undoubtedly also play an important role in protein and amino acid utilization.

In the early phases of the present investigations it became apparent that there would be need for a simple *in vitro* technique for estimating the susceptibility of protein, before and after heat-processing, to enzymic digestion. In this report are presented the *in vitro* digestibility method selected, the details and reliability of the improved formol titration procedure used in the *in vitro* studies, the variables requiring control in the application of the *in vitro* procedure to the determination of the susceptibility of protein to enzymic digestion, the results of the *in vitro* studies of the influence of heat-processing on protein digestibility, and the correlation of the *in vitro* findings with the results of biological and microbiological studies.

Experimental Part

Various methods are available for biological determination of the nutritional value of protein. The preferred technique is undoubtedly the nitrogen balance method of Mitchell and his associates *(53)*. The procedure affords a measure of the retention of absorbed nitrogen for tissue protein synthesis (the biological value of the protein) and at the same time gives a specific value for the percent of the dietary nitrogen that is absorbed (the degree or coefficient of protein digestibility). That the precision of the assay technique is excellent is indicated by the ease with which reproducibility can be held within $\pm$ 5 percent. However, the procedure demonstrates but does not explain why the same protein after various methods of processing shows differences in biological value even though the amino acid composition and coefficient of digestibility remain unchanged. It will be shown in this report that by supplementing the bioassay technique with an *in vitro* digestibility procedure, the probable basis for this phenomenon may be elucidated. Indeed, the method for determining the *in vitro* digestibility of a given food material, after correlation has been obtained with the bioassay has practical value in plant control of processing methods affecting the nutritional quality of the protein since it obviates the need for confirmatory bioassays.

The in vitro Digestibility Technique

The details of the *in vitro* technique for estimating susceptibility of proteins to enzymic digestion are presented in Figure 1.

A suspension or solution of the material containing six

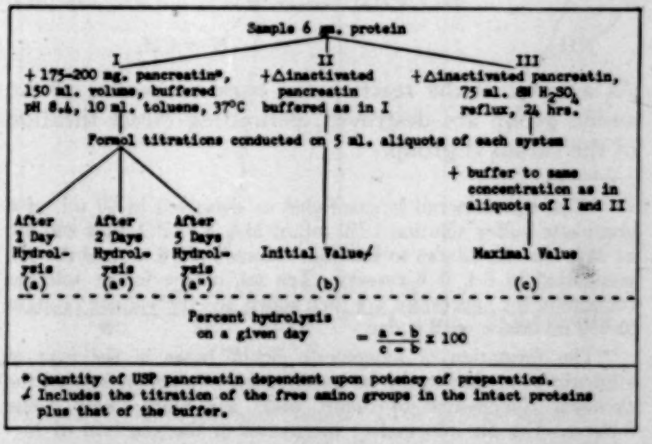

Sample 6 gm. protein
I
II
III
+ 175–200 mg. pancreatin*, 150 ml. volume, buffered pH 8.4, 10 ml. toluene, 37°C
+ Δinactivated pancreatin buffered as in I
+ Δinactivated pancreatin, 75 ml. 6N H_2SO_4 reflux, 24 hrs.
Formol titrations conducted on 5 ml. aliquots of each system
+ buffer to same concentration as in aliquots of I and II
After 1 Day Hydrolysis (a)
After 2 Days Hydrolysis (a^1)
After 5 Days Hydrolysis (a^2)
Initial Value/ (b)
Maximal Value (c)
Percent hydrolysis on a given day = $\frac{a-b}{c-b} \times 100$
* Quantity of USP pancreatin dependent upon potency of preparation.
/ Includes the titration of the free amino groups in the intact proteins plus that of the buffer.

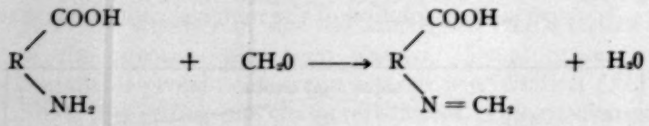
FIG. 1. *In vitro* technique for estimating susceptibility of proteins to enzymic digestion.

grams of protein is buffered [4] to pH 8.4, 175 to 200 mg. of USP pancreatin added, and the volume brought to 150 ml. Ten ml. of toluene are introduced and the mixture incubated at 37° C. Periodic formol titrations are conducted on 5 ml. aliquots of the enzymic digests. To another sample of the protein-bearing material, similarly buffered and diluted, is added the heat-inactivated pancreatin and an aliquot then taken for the formol titration. To a third sample of the test material, the sulfuric acid is added, and the suspension refluxed for 24 hours. A 5 ml. aliquot is taken, buffer added to the same concentration as in aliquots of I and II, and the material then analyzed by formol titration. In the case of III, the maximal titration value is obtained, representing ultimate hydrolysis to the amino acid stage. Thus (a — b), the increase of free amino nitrogen on a given day, over (c — b), the theoretical value for liberated amino nitrogen in a completely hydrolyzed sample, times 100 gives the percent hydrolysis of the protein. (It has proved desirable in many instances to determine the percent hydrolysis attained at intervals during the first 24-hour period). The use of sub-optimal quantities of the pancreatin permits digestion to proceed for several days at 37° C., slowing down the rate of liberation of the amino acids to such an extent that differences in digestibility of proteins may be detected. In materials containing readily digestible protein, hydrolysis is deliberately limited to approximately 50 percent. Obviously, this system does not duplicate conditions in the gastrointestinal tract. However, it constitutes a simple method for use as a routine laboratory control measure of the relative digestibility of the protein in materials undergoing heat-processing. Correlation of this simplified procedure with the results of animal assays to be presented in this report has amply justified the empirical conditions of the test.

The validity of the *in vitro* technique for determining protein susceptibility to enzymic digestion leans heavily on the reliability of the formol titration procedure.

The Formol Titration Procedure and Its Reliability

The oldest and simplest formulation [5] of the reaction between amino acids and formaldehyde is as follows:



As a result of the reaction the basic properties of the amino group are destroyed, permitting direct titration of the carboxyl group.

[4] The test material is suspended or dissolved in 60 ml. of a phosphate buffer solution (250 ml. of M/5 KH_2PO_4 plus 240 ml. of M/5 NaOH, diluted to 1000 ml. volume; pH 8.4), and the pH readjusted to 8.4, if necessary. Ten ml. of the buffer solution containing the pancreatin are then added and the volume brought to 150 ml. mark with water.

[5] The formation of monomeric Schiff bases in the case of aliphatic compounds may be questionable; compounds with the chemical composition of Schiff bases are more likely cyclic trimers. For an informative discussion of the reaction of formaldehyde with amino acids and proteins, reference is made to the excellent review article by French and Edsall *(24)*.

In carrying out the formol titration, the test solution, containing approximately 30 mg. of nitrogen, is first adjusted to pH 7.0 in a total volume of 20 ml. Two ml. of a 37 percent solution of formaldehyde, adjusted to pH 7.0, are added. This causes a decrease in the pH of the solution in most cases between 5.0 and 6.0. The solution is then titrated to pH 9.5 with standard O.1N sodium hydroxide. The formol titration as usually described is based on the use of a visual end-point, phenolphthalein being employed as the indicator. In the present procedure all pH measurements are made with the Beckman pH meter, using the glass electrode. Whereas the methods employed in the literature, almost without exception, terminate the formol titration at pH 8 to 9, it is specified here that the test solutions be titrated to pH 9.5.

The justification for this change in the procedure is evident from the titration curves in Figure 2. Here are plotted the percent of total nitrogen titrated at a given pH following the addition of the formaldehyde. It may be noted that at pH 9.5 the formol titratable nitrogen values equal the Kjeldahl nitrogen figures for ammonium chloride, for valine (a typical monoamino-monocarboxylic acid), and for glutamic acid (a typical monoamino-dicarboxylic amino acid). In the case of glutamic acid the adjustment of the solution to pH 7 neutralizes one carboxyl group so that subsequent titration, following the addition of formaldehyde, is similar to that for the monoamino-monocarboxylic acid. In the case of tryptophane and the basic amino acids (each of the latter as the hydrochloride), the formol titratable nitrogen values are lower. In valine there is one nitrogen per carboxyl group; in the others there is more than one nitrogen per carboxyl group. Thus for tryptophane containing two nitrogens the value calculated from the formol titration is found to be exactly one-half of the total Kjeldahl nitrogen; for arginine containing four nitrogens per carboxyl group the nitrogen by formol titration is exactly one-fourth; histidine with three nitrogens per carboxyl group gives a formol titratable nitrogen value 35 percent of the total nitrogen figure. On the other hand, lysine with two nitrogens gives a value 90 percent of the Kjeldahl value. Apparently in the case of lysine the hydrochloride group still remains attached to one of the amino groups when the solution is brought to pH 7, and this as well as the carboxyl group is titrated following reaction of formaldehyde with the amino groups. The basicity of the guanidine group in arginine is probably so great that the bound hydrochloric acid is not released following neutralization to pH 7.0, addition of the formaldehyde and subsequent titration. In the case of histidine, having an isoelectric point very much closer to that of the monoamino-monocarboxylic acids than that of either lysine or arginine, little hydrochloric acid remains bound after the pH adjustment to 7.0 to complicate the formol titration. In titrating the other amino acids selected, some discrepancies between formol titratable and total nitrogen values are found but these are not excessive.

It is apparent from the results obtained that if the titrations were terminated at pH 8 or 9, appreciably

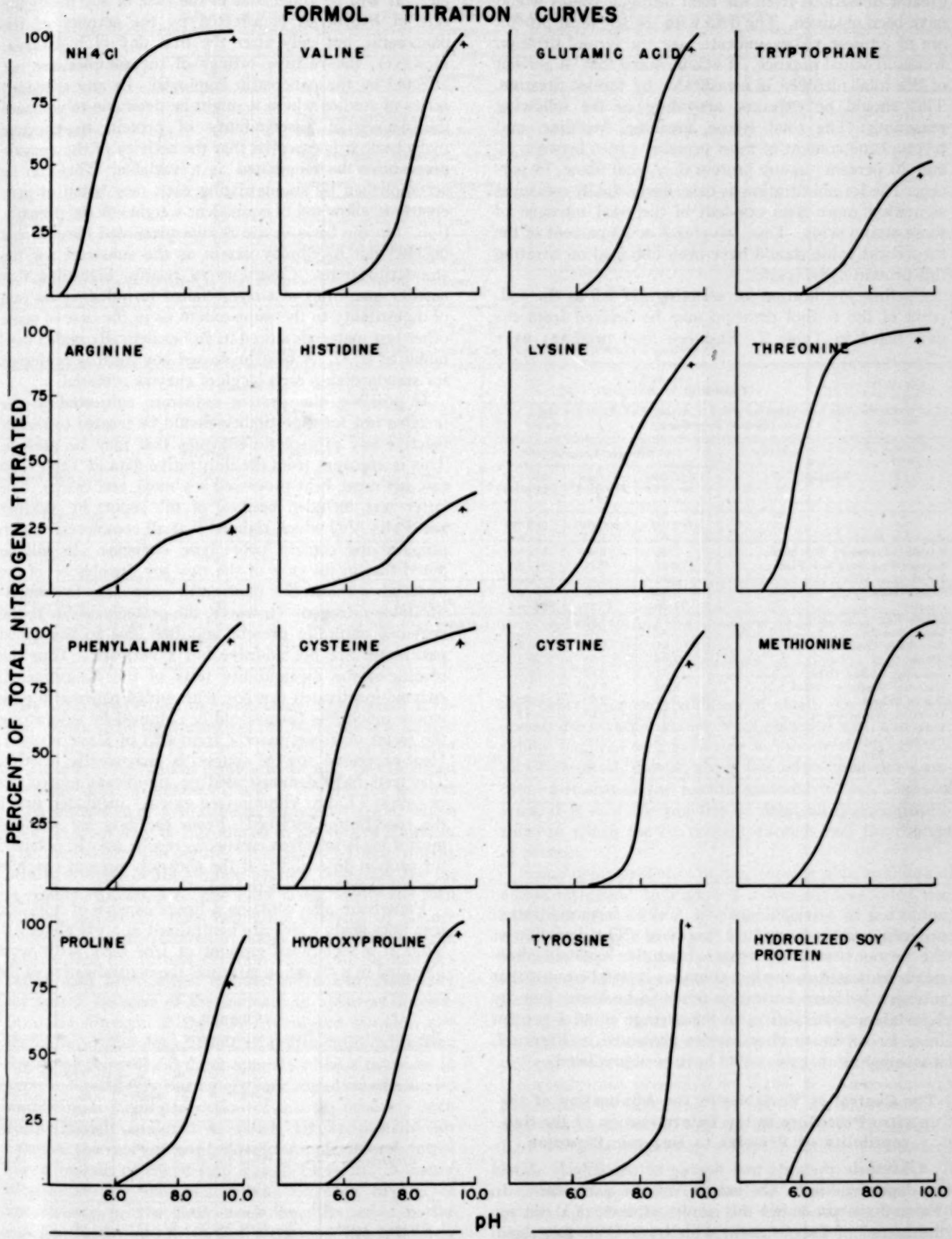


FIG. 2. Relation of formol titratable nitrogen figures at different pH values to total nitrogen content. (The arrows point to pH 95.)

greater deviations from the total nitrogen values would have been obtained. The data with the soy meal protein are of interest. They indicate how the formol titration works in actual practice. It will be noted that 94 percent of the total nitrogen is measurable by formol titration. This should be expected according to the following reasoning: The total lysine, arginine, histidine, and tryptophane content of most proteins varies between 10 and 20 percent; in soy protein they total about 14 percent. The formol titration as described actually measures somewhat more than one-half of the total nitrogen of these amino acids. Thus, 86 plus 7 or 93 percent of the theoretical value should have been obtained on titrating this protein hydrolysate.

Further justification for selecting pH 9.5 as the end-point of the formol titration may be derived from the data listed in Table 2. Eighteen food products were

TABLE 2

Justification for Selecting pH 9.5 As the End Point for the Formol Titration

Sample	Nitrogen Content		
	Kjeldahl Analysis (a)	Formol Titration* (b)	Deviation†
	percent	*percent*	*percent*
Raw Soy Meal	6.00	5.63	— 6.1
Expeller Processed Soy Meal	7.39	6.80	— 8.0
Solvent Processed Soy Flour	8.43	7.71	— 8.5
Dried Skim Milk	5.45	5.62	+ 3.2
Whole Milk	0.55	0.53	— 6.8
Casein	14.36	13.47	— 6.1
Whole Egg	1.92	1.89	— 1.7
Yeast Powder	7.40	7.40	0.0
Rice Bran Concentrate	1.41	1.41	0.0
Whole Oats	1.94	1.98	+ 2.5
Dehulled Cooked Oats	2.54	2.62	+ 3.1
Defatted Sunflower Meal	8.24	7.46	— 9.4
Whole Wheat	2.61	2.70	+ 3.4
Defatted Wheat Germ	5.54	4.67	— 15.6
Beef Steak	3.32	3.12	— 6.3
Chicken Product	2.40	2.46	+ 2.6
Liver Product	2.19	1.98	— 9.5
Whole Beef Blood	2.88	2.80	— 2.8
Average			— 3.6

*Of acid hydrolyzed sample.

† $\frac{b-a}{a} \times 100.$

subjected to Kjeldahl analysis and to formol titration according to the procedure described. The deviation of the formol titratable nitrogen from the Kjeldahl nitrogen is indicated in the last column. It will be noted that nitrogen by formol titration tends to be lower than by Kjeldahl anaylsis, being on the average about 4 percent less. From the titration curves presented in Figure 2, it is apparent that this could have been predicted.

The Control of Variables in the Application of the *in vitro* Procedure to the Determination of the Susceptibility of Proteins to Enzymic Digestion

Obviously the rate and degree of proteolysis should be dependent upon the activity of the pancreatin. In Table 3 are presented the results of tests of three soy meals and of Labco casein with fresh USP pancreatin and with a preparation which had been stored two years at 5° C. These two preparations had originally given the same formol titration values for the same test samples. It will be noted that in the case of soy meals the rate of hydrolysis is affected by the activity of the pancreatin, but only after the first day of hydrolysis. However, the relative ratings of the samples are not affected by the pancreatin employed. In any extended series of studies where it might be desirable to compare the degree of susceptibility of protein to enzymic hydrolysis, it is essential that the activity of the enzyme preparation be eliminated as a variable. This can be accomplished by standardizing each new batch of pancreatin to allow use of equivalent weights of the preparation. On the basis of the results presented here, it was decided not to employ casein as the substrate for the standardizations. Casein is so readily digestible that smaller quantities of enzyme failed to influence its rate of digestibility to the same extent as in the case of some other test materials stored in an hermetically sealed container at 0° C. A heat-processed soy meal is preferred for standardizing each batch of enzyme material.

If possible, the protein substrate subjected to the *in vitro* test for digestibility should be treated to render inactive any proteolytic enzymes that may be present. This is apparent from the illustrative data in Table 4 on raw soy meal, heat-processed soy meal, and casein. The latter was included because of the report by Warner and Polis *(74)* which claimed that all commercial casein preparations contain proteolytic enzymes. It will be noted that in the case of the raw soy, incubation of the material without the pancreatin gave rise to formol-titratable nitrogen. However, the proteolysis due to the enzymes naturally present and that due to the added pancreatin are not additive but synergistic. Thus the results of the digestitbility tests of the fat-extracted, enzyme-inactivated raw soy with added pancreatin gave values much less than would be obtained by subtracting the result of Experiment 2 from that of Experiment 1. Heat-processed soy, of course, is enzymically inactive. The tests have demonstrated no significant proteolysis of casein (Labco vitamin-free casein) incubated under the conditions described. From the data for raw soy meal it is obvious that erroneous results may be obtained for protein digestibility if the naturally present enzymes are not previously inactivated.

Pancreatin also contains a lipase capable of releasing free fatty acids which are neutralized at a pH above 7.0. Thus if a significant amount of free fatty acids were liberated in a digestion mixture, the neutralization of the

TABLE 3

*Rate and Degree of Proteolysis Dependent Upon Activity of Pancreatin**

Pancreatin	Test Sample	Degree of Hydrolysis of the Proteins		
		After 1 Day	After 2 Days	After 3 Days
		percent	*percent*	*percent*
Fresh Preparation	Soy Meal No. 1	8	15	18
	Soy Meal No. 2	12	19	28
	Soy Meal No. 3	12	19	33
	Casein	19	32	43
Stored 2 Years at 5° C.‡	Soy Meal No. 1	9	10	13
	Soy Meal No. 2	12	14	17
	Soy Meal No. 3	10	14	21
	Casein	20	31	37

*For each experiment 200 mg. of USP pancreatin was employed.

‡ Sample had been used repeatedly during this holding period, involving frequent removals from the refrigerator and exposure to the atmosphere.

TABLE 4

Influence of Naturally Occurring Proteolytic Enzymes on the In Vitro Test of Protein Digestibility

Sample (Protein Content)	Experiment		Hydrolysis of Proteins		
	No.	Description	After 1 Day	After 2 Days	After 5 Days
			percent	*percent*	*percent*
Raw Soy Meal* (38.3%)	1	Incubated with pancreatin	24	29	37
	2	Incubated without pancreatin	0	4	9
	3	Fat extracted, inactivation of enzyme at 100° C., then incubated with pancreatin	10	16	21
	4	Same as 3, but without pancreatin	0	0	0
Processed Soy Meal (49.0%)	5	Incubated with pancreatin	14	25	37
	6	Incubated without pancreatin	0	0	0
Casein (91.6%)	7	Incubated with pancreatin	19	32	43
	8	Incubated without pancreatin	0	0	0

*Digested samples subjected to ether extraction prior to formol titrations.

TABLE 5

Influence of Fat in Test Sample on the In Vitro Test of Protein Digestibility

Sample	Composition		Test	Enzymic Hydrolysis of Proteins			F. T. N.† in Acid Hydrolyzed Sample
	Protein	Fat		After 1 Day	After 2 Days	After 5 Days	
	percent	*percent*		*percent*	*percent*	*percent*	*% Kjeldahl Value*
Whole Milk	3.50	4.0	Direct	16	21	25	113
			Defatted*	20	25	28	93
Hard Boiled Egg	12.0	11.5	Direct	10	13	29	103
			Defatted*	10	14	31	98
Raw Soy Meal	38.3	21.8	Direct	9	16	20	100
			Defatted*	10	16	21	91
Expeller Processed Soy Meal	49.0	1.6	Direct	14	25	37	91
			Defatted*	14	25	37	91

* Enzymic and acid digests adjusted to pH 1, extracted with ether (residual solvent evaporated off), and then subjected to formol titrations.
† "Formol titratable nitrogen."

fatty acids might lead to erroneously high formol titration values. The significance of the fat in the test sample on the *in vitro* test of protein digestibility is presented in Table 5. The ratio of protein to fat may be calculated from the data in columns 2 and 3. When the amount of fat relative to protein is large, such as in egg or whole milk, an error may be introduced in the formol titration. The actual titration figures demonstrated that during *acid* hydrolysis of the protein-bearing materials, the fat is readily converted to free fatty acids which are then titrated. However, only negligible amounts of fat are hydrolyzed by the pancreatin under the conditions of the test, so that the formol titration values on the samples before and after ether extraction remain essentially the same. Because of the increase in apparent formol-titratable nitrogen in the acid-hydrolyzed samples, and therefore in the denominator in the fraction expressing degree of proteolysis, there appears to be a decrease in protein digestibility during enzymic digestion of samples containing a large proportion of fat. In products with approximately one-half as much fat as protein, for example the raw soy meal, no appreciable error is introduced by use of the direct formol titration. A factor responsible for the smaller-than-expected degree of interference of the fatty acids in this series is the rapidity with which formol titrations are routinely carried out. The fatty acids are insoluble in water and only by neutralization with sodium hydroxide are they brought into the aqueous phase. This involves stirring and delay after each addition of alkali. In the present series, the titration values were obtained with the same timing required for conducting formol titrations on samples free of fat, that is, about five minutes for the titration. Because of the results obtained in this series of tests, it is now the practice to defat all digestion mixtures in which the fat content exceeds half the content of protein.

Some plant proteins contain considerable amounts of amide nitrogen. In Figure 2 it was demonstrated that ammonia reacts like a free amino group in the formol titration. Amide nitrogen, on the other hand, fails to influence the titration values. However, in the acid hydrolysis of the protein for the theoretical maximum formol titration, amide nitrogen is converted to ammonia. The possibility therefore existed that some error might be introduced unless correction is made for ammonia derived from this source. This was investigated and the results are presented in Table 6. Ammonia was determined by adjusting the pH of the suspension to 12.0 and vacuum-distilling the ammonia at 35 to 40° C. into solutions containing a known volume of standard acid. It will be noted that there was no difference in the rate or degree of hydroylsis of the protein as a result of free ammonia in the digest, the figures with and without corrections for ammonia being essentially the same. This is explained by the fact that during enzymic hydrolysis of the protein, ammonia was liberated at almost the same rate as the amino groups. Thus,

TABLE 6

Influence of Ammonia Derived from Amide Nitrogen on the In Vitro Test of Protein Digestibility

Sample (Protein Content)	Amide Nitrogen	Test	Hydrolysis of Proteins		
			After 1 Day	After 2 Days	After 5 Days
	% of total N		*percent*	*percent*	*percent*
Defatted Sunflower Meal (52.5%)	17	Direct	16	25	37
		Correcting for NH_3 in spls.	17	28	34
Yeast Powder (46.2%)	10	Direct	21	30	36
		Correcting for NH_3 in spls.	23	32	36
Dried Skim Milk (32.8%)	6	Direct	29	33	35
		Correcting for NH_3 in spls.	34	35	39
Hard Boiled Egg (12.0%)	6	Direct	11	16	31
		Correcting for NH_3 in spls.	10	14	31
Expeller Processed Soy Meal (49.0%)	1	Direct	14	25	37
		Correcting for NH_3 in spls.	14	25	37

although the actual titration values are somewhat different, particularly in the case of the sunflower meal where appreciable amounts of amide nitrogen are found, the over-all picture of digestion of the protein remains the same.

Another point worthy of mention is the effectiveness of the antiseptic in the enzymic digestion mixture. Toluene has been employed for this purpose in the current studies. Tests conducted on whole beef blood, a favorable medium for the propagation of many types of bacteria, have indicated that toluene is very effective as a bacteriostatic and even as a bactericidal agent. The use of toluene and benzene in microbiological assays when test materials are incubated with enzymes for the liberation of the bound forms of vitamins is, of course, common practice. When ineffective bacteriostatic agents were employed, putrefaction occurred in the digestion mixtures, and the formol titration values were as much as 30 percent greater than the figures for the acid hydrolyzed samples. This was due to acid decomposition products which are also measured in the formol titration.

With all the factors mentioned under control, Table 7 presents data on the reproducibility of the technique for estimating the susceptibility of proteins to *in vitro* enzymic digestion. For each experiment 200 mg. of USP pancreatin of the same batch was employed. These duplicate series of enzymic digestions were carried out at different periods, some spaced as much as one month apart. The data show that the reproducibility of the test procedure is excellent. Insofar as the formol titration itself is concerned, the precision attainable is similar to that of the usual acidimetric-alkalimetric titrations.

TABLE 7

*Reproducibility of Technique for Estimating Susceptibility of Proteins to Enzymic Digestion**

Sample	Protein Content (N x 6.25)	Degree of Hydrolysis of the Proteins		
		After 1 Day	After 2 Days	After 5 Days
	percent	*percent*	*percent*	*percent*
Casein	91.6	18	30	41
		19	32	43
Solvent Processed Soy Flour	52.7	8	9	11
		8	12	14
Expeller Processed Soy Meal	46.2	13	23	31
		14	21	33
Defatted Sunflower Meal	51.4	12	26	38
		16	25	37
Whole Oats	14.3	19	26	30
		17	22	28

* For each experiment 200 mg. of USP pancreatin of the same batch was employed.

In vitro digestibility studies

The first application of the *in vitro* protein digestibility test was made to soybean products which had undergone heat-processing to improve their nutritive value. This type of material is well suited for studying the validity of the hypothesis that rate of digestibility is an important factor in determining the utilization of the amino acids, since it shows no difference in amino acid composition or coefficient of digestibility before and after various types of processing, but exhibits marked differences in biological value. The results of two series of digestibility studies are presented in Table 8.

TABLE 8

Susceptibility of Soy Proteins to Enzymic Digestion

Sample (Protein Content)	Laboratory Processing	Hydrolysis of Proteins		
		After 1 Day	After 2 Days	After 5 Days
		percent	*percent*	*percent*
Solvent Processed Soy Flour (52.7%)	None	8	9	11
	△ 100° C. 7 days, atmospheric pressure	8	9	11
	△ in water, 100° C. 5 min., atmospheric pressure	7	9	11
	Autoclaved 10 min. at 5 lbs. pressure	9	10	14
	Autoclaved 10 min. at 10 lbs. pressure	12	21	24
	Autoclaved 30 min. at 10 lbs. pressure	14	23	29
Expeller Processed Soy Grits (48.7%)	None	16	20	24
	Autoclaved 10 min. at 10 lbs. pressure	20	23	29
	Autoclaved 30 min. at 10 lbs. pressure	22	27	33
	Autoclaved 30 min. at 15 lbs. pressure	18	25	34
	Autoclaved 45 min. at 15 lbs. pressure	16	25	33

Dry heating of soy flour at 100° C. at atmospheric pressure results in no improvement in the biological value of the protein. The same is generally conceded if the material is heated for a short period of time in boiling aqueous suspension. However, heating with superheated steam or autoclaving results in an increase in the biological value of the protein *(34, 62, 63, 49, 25, 77)*. In this table are presented the results of tests of two soy products, one a solvent-processed soybean flour, the other an expeller-processed soy grit. The solvent processing of soy flour, extraction with a fat solvent at moderately low temperatures (in the neighborhood of 58° C.) is not very effective in increasing the biological value of the protein. On the other hand, the expeller process for obtaining fat from soybeans involves the building up of a high internal temperature in the pack so that the protein receives effective heat treatment at the same time the fat is being expelled. It will be noted from the data in Table 8 that those conditions which fail to affect the biological value of the soy protein also fail to increase the digestibility of the soy protein. Thus, the solvent-processed soy when heated at 100° C. either in the dry state or in aqueous suspension exhibits no improvement in rate and degree of digestibility of the protein component. As the material is autoclaved there is a progressive increase in its digestibility concomitant with the increase in biological value. In the case of the soy grits improved biological value is accompanied by greater degree and rate of digestibility of the untreated sample. Here, too, an increase in digestibility of the protein occurs as a result of the autoclaving. This soon plateaus out, hence there is no advantage in autoclaving beyond a limited period or at increased pressure. Inasmuch as the availability of certain of the amino acids may be reduced by excessive heat treatment, it may be found that at some point before maximum digestibility is obtained the product possesses maximum biological value. Almquist and collaborators *(2)* and Hayward and his group *(32)* have demonstrated that the limiting amino acid in soy protein is methionine and that autoclaving increases the availability of this amino acid. But precautions must be taken lest the effort to increase the availability of methionine be accompanied by decreased availability of other amino acids, notably lysine.

In Table 9 are listed the results obtained in studies of the influence of processing on the susceptibility of other proteins to *in vitro* enzymic digestion. The marked increase in the digestibility of the protein in whole egg after heat coagulation is in agreement with the reports in the literature *(3, 12, 31)*. The protein in whole beef blood shows a similar improvement in digestibility; in the case of the milk proteins the same is noted but to a lesser degree. Heating whole oats under steam pressure appears to be without effect on digestibility. Tests conducted on samples autoclaved for shorter and longer periods of time gave essentially the same results as the untreated samples. In the case of the defatted wheat germ there is a decrease both in degree and in rate of enzymic hydrolysis of the protein after the autoclaving treatment. The significance of these changes on the nutritive value of the proteins can be ascertained only by correlation with animal assays. In view of species differences in biological responses, recently noted *(13)* in protein utilization studies, precautions must be observed in drawing broad generalizations.

TABLE 9

Influence of Processing on the Susceptibility of Proteins to Enzymic Digestion

Sample	Processing	Hydrolysis of Proteins		
		After 1 Day	After 2 Days	After 5 Days
		percent	*percent*	*percent*
Whole Egg	Raw	1	8	12
	Hard Boiled	11	16	31
Milk	None	19	24	28
	Skimmed and spray dried	29	33	35
Whole Oats	None	19	26	30
	Autoclaved 30 min. at 15 lbs. pressure	17	22	27
Defatted Wheat Germ	None	29	38	52
	Autoclaved 30 min. at 15 lbs. pressure	16	29	38
Beef Blood	None	4	8	16
	Proteins heat coagulated	31	47	60

Biological and Microbiological Studies of Food Proteins Improved in Nutritive Value by Heat-Processing

The remainder of this paper is concerned with animal and microbiological assays designed to test the validity of the hypothesis dealing with the importance of the rate of digestibility of proteins *in vivo*. The soybean has been selected as the prototype of those proteinaceous products whose nutritional value is improved by heat-processing.

In Table 10 is a summary of the data obtained in correlating the *in vitro* tests of protein digestibility with rat growth studies. The soy products, the control milk sample, and the bioassay values were kindly supplied by Dr. J. W. Hayward of the Archer-Daniels-Midland Company. He has expressed the relative protein efficiency of the soy samples as the weight gained per gram of protein consumed, assuming an efficiency of 100 percent for milk proteins. It will be observed that the extracted and *toasted* soy meal was superior to the expeller soy meal, which in turn was superior to the raw soy meal. His studies also demonstrated that these differences in protein efficiency were not correlated with the coefficient of digestibility of the proteins. It will be noted that the *in vitro* digestibility tests of the

TABLE 10

Correlation of the In Vitro Test of Protein Digestibility With Rat Growth Studies

Sample*	*In Vitro* Hydrolysis of Proteins			Protein Efficiency In Rat Assay†
	After 1 Day	After 2 Days	After 5 Days	
	percent	*percent*	*percent*	*percent*
Dried Skim Milk	29	33	35	100
Extracted and Toasted Soy Meal	17	34	46	86
Expeller Soy Meal	13	23	31	71
Raw Soy Meal	12	19	26	53

* Samples and bioassay values kindly supplied by Dr. J. W. Hayward of Archer-Daniels-Midland Co., Mineapolis. The meals were from the same batch of soybean.

† As weight gained per gram of protein consumed, expressed relative to the milk protein having an assigned value of 100%.

soy meals show the very same relationship as the animal assays. However, comparisons should not be made with the results obtained with the dried skim milk since the amino acid composition of the protein in the latter is different from that of soy protein. The *in vitro* digestibility data should be compared only in respect to an individual protein material to ascertain the influence of processing or storage for extended periods of time.

In Table 11 is a correlation of the results of *in vitro* tests of soy protein digestibility with nitrogen balance studies on rats, using for the latter the procedure of Mitchell and associates *(53)*. No. 6 was the raw soy meal; No. 5 the solvent-extracted meal; the others were heat-processed. All the meals were prepared from the same batch of soybean. It will be noted, in considering the data obtained with meals No. 1 through 6, that the decrease in rate and degree of protein digestibility *in vitro* is accompanied by a decrease in the biological value, while the coefficient of digestibility of the protein is not significantly affected. In other words, the heat-processing fails to make available (absorbable) a greater amount of protein nitrogen for the rat to use in the building of new tissue protein. However, the percentage of absorbed nitrogen retained for tissue protein anabolism was much greater in the case of the processed soy No. 1 as compared with the raw meal No. 6.[†] It would seem that the protein in soy meal No. 1, by virtue of being much more readily digestible, liberates methionine (the limiting amino acid) at a more rapid rate and at a more opportune time in relation to the other amino acids which it supplements. In the case of the raw soy, the methionine may be liberated too late to supplement the amino acids already absorbed. Thus, not only is the methionine inefficiently utilized for protein synthesis, but other amino acids, which require methionine supplementation, are likewise poorly utilized, being converted to energy rather than tissue substance.

On the other hand, it might be assumed that the coefficient of digestibility, dropping from 84 to 81, would be of very great importance if the difference of 3 percent represented principally failure of methionine absorption. In that case, the differences in the biological values could be due simply to the degree rather than to the rate of methionine liberation *in vivo*. However, the hypothesis in the present study requires that the methionine in the raw soy be absorbed to almost the same extent as the methionine in the processed soy, but after too long a time interval effectively to supplement the other amino acids previously absorbed.

Because of these conflicting interpretations, specific tests were designed to study the problem. These involved studies of methionine absorption by the rat consuming the test rations. In conducting the nitrogen balances according to the technique described by Mitchell *(53)*, it is necessary to feed the animals a low protein diet containing lactalbumin, at a level of about 3 to 4 percent of the ration, before and after the actual assays with the test proteins. In the course of these successive assay periods a dry skim milk sample is included, so that comparisons may be drawn between the biological value and coefficient of digestibility of the test samples with that of whole milk protein. The results of the methionine balance studies are summarized in Table 12.

It will be noted that despite an increased methionine intake of 250 percent by the feeding of the milk proteins, the same fecal excretion of this amino acid occurred. This is good evidence that the 70 mg. of methionine

TABLE 11

Correlation of the In Vitro Test of Protein Digestibility With Nitrogen Balance Studies on the Rat: Studies With Soy Protein

Soy Meal*	*In Vitro* Hydrolysis of Proteins			Rat Assays of Proteins	
	After 1 Day	After 2 Days	After 5 Days	Biological Value	Coefficient of Digestibility
	percent	*percent*	*percent*	*percent*	*percent*
1	14	25	37	71	84
2	13	22	33	67	85
3	14	22	32	66	82
4	11	17	23	64	84
5	10	16	21	61	81
6	11	16	22	53	81
7	11	15	24	72	88

* Sample No. 6 was the raw soy meal; No. 5 the solvent extracted meal; the others were heat-processed. All the meals were prepared from the same batch of soybean.

[†] The results with soy meal No. 7 are included in Table 13, although it is an exception to the general rule. The product was a heat-processed preparation, quite brown in color, with as high a biological value as soy meal No. 1, but it exhibited relatively poor digestibility *in vitro*. It may be that substances are present in this product as a result of the processing, inhibitory of enzymic activity *in vitro* but not *in vivo*.

TABLE 12

Methionine Absorption by the Rat Consuming Diets Containing Milk or Soy Protein

Ration*		Intake of Methionine	Fecal Excretion of Methionine	Unabsorbed Methionine		Assay of Proteins	
Description	Protein Content			Corrected‡	Part of Intake	Biological Value	Coefficient of Digestibility
	percent	*mg.*†	*mg.*†	*mg.*†	*percent*	*percent*	*percent*
Lactalbumin	2.96	61	65	0	0	....	....
Lactalbumin	2.96	65	69	0	0	....	....
Dried Skim Milk	8.50	225	75	0	0	89	84
Processed Soy—1	9.06	161	146	76	47	71	84
Processed Soy—2	9.09	178	150	80	45	67	85
Processed Soy—3	9.10	141	129	59	42	66	82
Processed Soy—4	9.24	200	147	77	38	64	84
Extracted Soy—5	9.30	158	133	63	40	61	81
Raw Soy—6	8.99	154	145	75	49	53	81

* The soy meals were all from the same batch of soybean.

† Per rat test period of 7 days.

‡ Correction is made for the metabolic fecal methionine, 70 mg. per rat per period. The same group of six adult rats was employed throughout the balance studies.

excreted per rat during the 7-day test period represented metabolic fecal methionine and not methionine derived from dietary protein. The processed, extracted, and raw soy meals were all fed at a protein level of approximately nine percent. Correction of the total fecal methionine for the metabolic excretion of methionine gives an index of the quantity of this amino acid which failed to be absorbed. Expressing this in terms of methionine intake, it was found that as much as 38 to 49 percent of the methionine in soy products was not absorbed from the gastrointestinal tract of the rat. Furthermore, this absorption of methionine showed no correlation with the biological value. The values for unabsorbed methionine were essentially the same considering the assumption of a constant metabolic excretion. The differences in biological value are significant since the values were reproducible within several percent. Johnson, Parsons, and Steenbock *(39)* have reported negligible decreases in fecal sulfur and nitrogen when raw soy meal was replaced with a properly cooked product. However, greater retention (decreased urinary excretions) of both these factors was noted when the heat-processed soy meal was fed. These observations are in complete harmony with the hypothesis presented in this report since they, too, indicate no decrease in methionine absorption when the raw soy is ingested, but poor utilization of both this and the other amino acids. More recently, Evans *(19)* reported no correlation between the "digestible" organic sulfur content of soy protein and its nutritional value.

The present experiment constitutes a specific case where degree of digestibility of the protein in the gastrointestinal tract as well as the extent of absorption of the limiting amino acid, methionine, are unchanged, but nevertheless, the biological value (retention of absorbed nitrogen) varies considerably. Apparently the methionine in the raw meal is absorbed so late during the gastrointestinal journey that this amino acid, as well as the incompletely supplemented amino acids, are not efficiently utilized for the synthesis of body protein.

The validity of this interpretation is supported by further experimental findings, presented in Table 13. The test materials were the raw soy and the processed meal with the highest biological value. The biological values and coefficients of digestibility of both products are listed. The percent of dietary methionine unabsorbed is also indicated. According to the *in vitro* test, one is more readily digested than the other. The proteins of both samples were then analyzed for three amino acids, leucine *(70, 48)*, lysine *(16)*, and methionine *(47, 1)*. The values are essentially the same for the two products and agree with those reported in the literature for soy protein. The digests obtained, in the course of the *in vitro* enzymic hydrolysis of the protein, at the end of the first and fifth days were also subjected to amino acid analysis. For this purpose trichloroacetic acid was added to the digest to make a 7 percent concentration. The filtrates were then heated to convert the trichloroacetic acid to chloroform and carbon dioxide which were volatilized from the boiling solutions. These were then subjected to acid hydrolysis to convert peptides and possibly larger molecules to the free amino acids. Thus the values for leucine, lysine, and methionine in the filtrates of the protein digests include not only these amino acids in the free state but also in peptide linkage not precipitable by 7 percent trichloroacetic acid. Obviously the percentage amino acids so liberated would be considerably greater than that estimated by formol titration which is a measure of only the free amino groups.

Methionine was liberated very slowly from raw soy as compared to leucine, while lysine was most rapidly released by enzymic digestion. In the case of the processed soy, the same relationship held, although all amino acids were released at a somewhat faster rate. When the five-day digests were tested, it was found that almost all the lysine and more than half of the leucine had been liberated. In the hydrolysate of the raw soy meal only 36 percent of the methionine was found, while in the digest of the heat-processed meal there was 73 percent. These data indicate that methionine is liberated from soy proteins at a slower rate than the other two amino acids, and that heat-processing of this protein increases the rate of liberation of the methionine to a much greater extent than of leucine or lysine.

Biological and Microbiological Studies of Food Proteins Impaired in Nutritive Value by Heat-Processing

Earlier in this report it was pointed out that excessive heat-processing reduces the biological value of protein without affecting materially the protein content, the essential amino acid composition, or the degree of protein digestibility *in vivo*. In such cases lysine has been found to be the principal limiting amino acid. These findings are comparable to those noted with raw foods, improved in protein value by heat-processing, with the

TABLE 13

Rate of Enzymic Liberation of Amino Acids During the In Vitro Test of Protein Digestibility As A Critical Factor in Determining Nutritional Response

Experiment	Raw Soy Meal (No. 6)	Processed Soy Meal (No. 1)
Rat Assays		
Biological Value of the Proteins, %	53	71
Coefficient of Digestibility of the Protein, %	81	84
Dietary Methionine Unabsorbed, %	49	47
In Vitro* Enzymic Hydrolysis of Proteins		
After 1 day, %	11	14
After 2 days, %	16	25
After 5 days, %	22	37
Amino Acid Composition of the Proteins†		
Leucine, %	7.1	7.7
Lysine, %	5.1	5.4
Methionine, %	2.2	2.2
Liberation of Amino Acids‡		
Leucine	28	33
Lysine	45	56
Methionine	5	10
%, After 1 Day Hydrolysis		
Leucine	52	65
Lysine	77	84
Methionine	36	73
%, After 5 Days Hydrolysis		

* Based upon liberation of free amino groups, as determined by formol titration.

† Analyses on acid hydrolyzed samples, values calculated to 16% nitrogen.

‡ Includes not only free amino acids but also peptides, liberated during the *in vitro* enzymic hydrolysis and not precipitated by 7% trichloroacetic acid. The values listed are percentages of the total.

exception that methionine is the limiting amino acid in the raw products. This portion of the report is concerned with an investigation designed to determine whether the same mechanism is responsible for the reduced biological value of excessively heat-processed protein; namely, impairment in the susceptibility of the protein to enzymic digestion with the result that the limiting amino acid is released *in vivo* too late to supplement the amino acids already absorbed.

The results of tests with two samples of dried skim milk are of interest and are present in Table 14. These samples were obtained from the same processor at two periods one year apart. The amino acid composition was, of course, the same, and yet when tested on rats the samples showed an appreciable difference in biological value, 78 percent as compared to 90 percent, but no significant difference in coefficient of digestibility, that is, the fraction of dietary protein absorbed from the gastrointestinal tract. However, the more readily digestible sample, according to the *in vitro* test, exhibited the higher biological value.

In subsequent studies designed specifically to determine what mechanism is responsible for the poor utilization of lysine in excessively heat-processed protein, casein was selected as the test material. In confirmation of the work of others *(5, 17)*, it has been found (see Table 15) that dry-heating the sample[g] at elevated temperature (150° C.) does not destroy the limiting amino acid, lysine. When the samples were subjected to maximal enzymic hydrolysis (peptic followed by pancreatic digestion), it may be noted (see Table 16) that the degree of protein hydrolysis was not affected by the heat treatment and that the microbiologically available lysine *(72)* content was not substantially reduced.

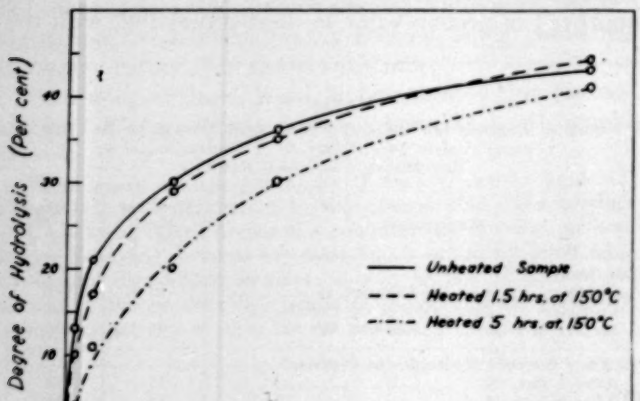


FIG. 3. Rate of *in vitro* pancreatic hydrolysis of casein samples. (From Pader, M., Melnick, D., and Oser, B. L. *J. Biol. Chem.*, 172, 763 [1948].)

In contrast to the above findings, striking differences in the *susceptibility* of the heated and unheated casein samples to *in vitro* pancreatic digestion are noted *(61)*. The data plotted in Figure 3 indicate that the heated protein samples are digested at significantly slower rates than the unheated control sample and that the rate of liberation of formol titratable nitrogen varies inversely with the processing time. The differences are of greatest relative magnitude during the early phase of hydrolysis. The ultimate degree of digestion of both the heated and unheated products eventually approaches the same value, in agreement with the results of the serial enzymic digestion experiments (see Table 16).

The differences in the *rate* of liberation of the available lysine from the casein samples are even more striking. Reference to Figure 4 shows that microbiologically available lysine was liberated from the heat-treated protein by pancreatin at a much slower rate than from the unheated product. The curves relating liberation of available lysine to time are linear up to a point at which they form a plateau. The relative slopes of the linear portions of the curves are as follows: 1, arbitrarily assigned to the control unheated sample; 0.66, casein heated 1.5 hours; and 0.25, sample heated 5 hours. Thus, substances with lysine activity are released from the unheated protein during the early period of digestion at a rate four times as great as from the sample submitted to prolonged heating, and 1.5 times as rapidly as from the intermediate preparation.

It is apparent that the differences in the content of microbiologically available lysine in the acid or final enzymic digests of heated and unheated casein are insufficient to account for the markedly reduced biological value of the heated protein. The results of the studies of

TABLE 14

Correlation of the In Vitro Test of Protein Digestibility With Nitrogen Balance Studies on the Rat: Studies With Milk Protein

Dried Skim Milk*	*In Vitro* Hydrolysis of Proteins			Rat Assays of the Proteins		
	After 1 Day	After 2 Days	After 5 Days	Series	Biological Value	Coefficient of Digestibility
	percent	*percent*	*percent*	*percent*	*percent*	*percent*
1	27	31	33	I	77	89
				II	79	87
2	32	36	41	I	90	85
				II	89	84

* Samples obtained from the same processor.

TABLE 15

The Lysine Content of the Test Casein Samples

Casein Sample	Lysine Found*
	percent
Unheated	8.1
Heated 1.5 hours at 150° C.	7.9
Heated 5 hours at 150° C.	7.6

* Determined microbiologically after complete acid hydrolysis; values calculated to the pure protein basis, 15.4 percent nitrogen.

TABLE 16

*Enzymic Liberation of Lysine for Microbiological Utilization**

Casein Sample	Degree of Hydrolysis**	Microbiologically Available Lysine†	Lysine Utilized‡
	percent	*percent*	*percent*
Unheated	57	6.5	80
Heated 1.5 hours at 150° C.	51	5.9	75
Heated 5 hours at 150° C.	57	5.2	68

* Peptic digestion followed by pancreatic digestion *(61)*.
** Estimated by formol titration.
† In the final enzymic digest, calculated to the original protein basis.
‡ Percent of total lysine in casein samples.

[g] The casein sample was obtained from the S. M. A. Corporation, Chagrin Falls, Ohio.

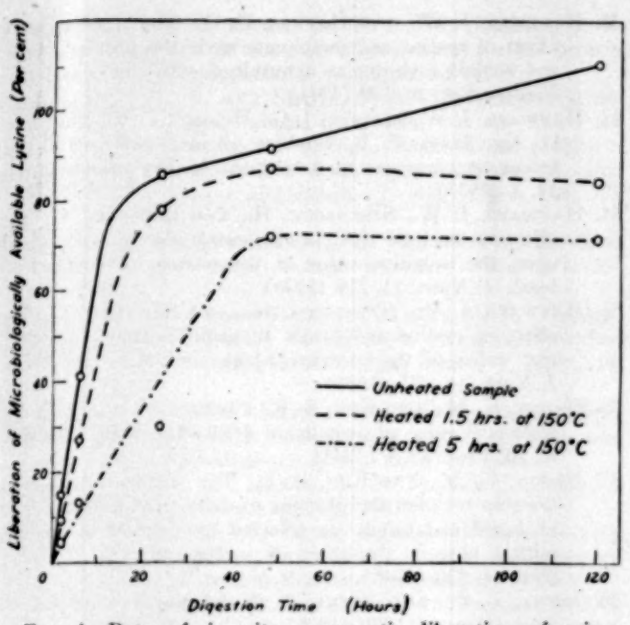


FIG. 4. Rate of *in vitro* pancreatic liberation of microbiologically available lysine from casein samples. (From Pader, M., Melnick, D., and Oser, B. L. *J. Biol. Chem.*, **172**, 763 [1948].)

the rate of liberation of lysine for microbiological utilization provide a plausible explanation for the impairment of the biological value. The rate of release (and absorption) of lysine from heat-processed casein is too slow to permit effective supplementation of the other essential amino acids *in vivo* with the result that nitrogen retention for tissue protein synthesis is impaired; hence the need for supplementing the heated casein with lysine for optimal utilization. Indeed, from the work of others *(64)* the lysine in excessively heat-processed protein may be released so late during the gastrointestinal journey that a large fraction may escape absorption.

Biological Studies of Food Proteins Whose Nutritive Value Is Not Affected by the Heat-Processing Employed

The proteins in excessively heat-processed cereals are recognized to exhibit little nutritive value *(56, 44, 58, 71, 43, 64)*. However, when these are properly cooked no undesirable changes in the protein component occur. In fact the protein in the unheated products appear to be equal in nutritive value to those cooked for improved palatability *(65)*.

In studies, summarized in Table 17, of various oat samples(raw, pan-toasted, and thoroughly cooked at atmospheric pressure), no significant differences in the digestibility of the protein *in vitro* were noted. All four products were rapidly hydrolyzed and reached a maximum degree of digestibility according to this type of test. The lack of effect of the heat-processing on the digestibility of oat protein is also evidenced by the uniformity in its biological value as determined by the nitrogen balance experiments. The somewhat greater value of 81 percent for the whole oats may be attributed in part to a difference in the quality of the protein consumed since the protein in this sample comes from the bran as well as the endosperm.

TABLE 17

Correlation of the In Vitro Test of Protein Digestibility With Nitrogen Balance Studies on the Rat: Studies With Oat Protein

Oat Sample*	*In Vitro* Hydrolysis of Proteins			Rat Assays of Proteins	
	After 1 Day	After 2 Days	After 5 Days	Biological Value	Coefficient of Digestibility
	percent	*percent*	*percent*	*percent*	*percent*
Whole Oats	27	40	51	81	85
Hulled and "Pan Toasted"	25	34	44	74	87
Cooked	18	36	53	75	88
Cooked	20	40	47	79	89

* From the same batch of oats.

Summary and Conclusions

The concept of food protein as N × 6.25 should be extended to include a consideration of both the functional and nutritive properties of the protein. The functional properties, readily modified by heat-processing, affect appearance and palatability of the end-product and for this reason cannot be dissociated from the nutritive properties of the protein. The functional properties of the protein are usually evaluated by subjective utility tests; it has been suggested that these be supplemented with precise objective procedures for determining the degree of protein denaturation caused by the heat-processing.

The nutritive properties of protein may be determined by animal assays but here also the need to supplement the data with results of *in vitro* tests has been emphasized. Determinations of the essential amino acid composition of the protein and its susceptibility to enzymic digestion contribute to an explanation of results obtained by animal assays. A simple *in vitro* procedure for determining the susceptibility of protein to enzymic digestion has been presented in this report. It has been shown in studies with products, improved or impaired by heat-processing and exhibiting no change in amino acid composition or degree of digestibility, that rate of enzymic digestion is critical. For optimal utilization of food proteins all essential amino acids must not only be available for absorption but must be liberated during digestion *in vivo* at rates permitting mutual supplementation.

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The Effect of Acid Foods on Aluminum Cooking Utensils[a]

CHARLES F. POE and JANETT M. LEBERMAN
Division of Sanitary Chemistry, University of Colorado, Boulder

The effects of acid foods on aluminum cooking utensils have been determined. Rhubarb and cherry juices dissolve the most aluminum from the utensils. The juices have less effect on wrought aluminum utensils than on cast aluminum utensils. Acidity is an important factor in the action of acid juices on aluminum utensils.

Introduction

A previous publication *(13)* presents the effects of dilute acids, which are usually present in fruits and vegetables, on strips of aluminum and on aluminum cooking utensils. Of the 0.1 normal organic acids used, trichloroacetic, acetic, formic, monochloroacetic, and lactic were the most active, in the order named, when the solutions of acids were boiled 30 minutes daily in contact with the aluminum. Oxalic acid, in normal solution, was very active at a temperature of 25° C. The present communication records the corrosive effect of fruit juices on various brands of commercial aluminum cooking utensils.

Many investigations have been made on the toxicity of aluminum added to food products. The general conclusion has been that small amounts have no detrimental effect in the body. These conclusions are confirmed in leading books on toxicology *(6, 9, 16)*. The Referee Board appointed by President Theodore Roosevelt in 1907 *(15)* reports that aluminum in baking powder, furnishing a daily intake up to 150 mg. of aluminum, contributed no poisonous or other deleterious effects which might be injurious to health.

The effects of acid foods on aluminum utensils have received some attention. Glaister and Allison *(8)* report that the amounts of aluminum dissolved by orange,

[a] Presented at the Detroit meeting, A. C. S. September, 1940.

lemon, and tomato products are extremely small. Blough *(3)* comes to the same conclusion when tomato products are cooked in aluminum. Blount and Bailey *(4)*, Rohrig *(14)*, and Mrak and Cruess *(11)*, all conclude that juices of citrus fruits have rather great effects on aluminum. Colobraro *(5)* states that tomato products dissolve considerable amounts from aluminum utensils. Beal, Unangst, Wigman, and Cox *(1)*, and Fillinger *(7)* report that acid foods dissolve very small amounts of aluminum.

Procedure and Methods

Twelve different brands of aluminum cooking utensils were used in this research. Of these, five were made from cast aluminum and seven were made from wrought aluminum. These utensils, representing standard brands of aluminum cooking pans, were purchased on the open market. Aluminum used to make cooking utensils usually contains from 97 to 99 percent aluminum. The percentages of such elements as copper, silicon, magnesium, iron, and zinc sometimes are present in a few tenths percent each. Manganese may be present in amounts up to 1.0 percent.[b]

Cherry, orange, tomato, and rhubarb juices were prepared from the fresh fruits. The cherries were washed, the stems removed, and the fruit was boiled in a covered porcelain dish. The juice was extracted by means of a small hand juice-extractor, and was then strained through cotton. The oranges were reamed by hand, and the juice was strained before it was used. The tomatoes were cut into small wedge-shaped pieces and cooked in a covered porcelain dish. The juice and pulp were passed through a fine rotary sieve to separate them from the skin and seeds. The rhubarb was cut into small pieces, cooked in a porcelain dish with a small amount of water, and pressed. The added water was evaporated during the cooking process.

The sweetened canned fruit juices used were pear, plum, peach, loganberry, grapefruit, orange, pineapple, apricot, and prune. The unsweetened canned juices used were orange, grapefruit, pineapple, grape, apple, and yellow tomato.

Into each of the aluminum pans was placed a measured amount of a given juice. The contents of each container were heated directly over a flame to the boiling point, and then allowed to simmer on the steam bath for 30 minutes. After the heating process was completed, the volume was adjusted to the original amount with distilled water. Fifty-ml. amounts of each juice were measured into platinum dishes, evaporated to dryness, charred and ashed in the muffle furnace at a temperature not to exceed 650° C. A blank was run on each sample of juice in order to determine the amount of aluminum in the original juice. A single sample of juice was used for the tests in each of the different utensils. After cooking the juice in a given utensil, the pan was cleaned with fine steel wool.

The method used for the determination of aluminum was the official method for iron and aluminum in plant material as given by the Association of Official Agricultural Chemists *(12)*. Duplicate determinations were made in every instance.

The increased amounts of aluminum found in the juices, after being cooked in aluminum utensils, were calculated to parts per million. The results obtained, when wrought aluminum utensils were employed, are given in Table 1. Table 2 records the results in parts per million of aluminum dissolved by fruit juices in cast aluminum utensils. Table 3 records the relative acidities of the fruit juices used.

The results given in Table 1 show that rhubarb, cherry, prune, loganberry, and yellow tomato juices dissolved the most aluminum from the wrought aluminum utensils. Pear, apricot, peach, and pineapple juices proved to be the least effective on these utensils. All of these latter juices contained added sugar. Utensils numbers 6 and 8 were most affected by the different juices, and number 2 the least affected. According to the retailer, pans numbers 1 and 3 were of the same wrought aluminum composition, but sold under different trade names. The amounts of aluminum dis-

[b] Personal communication, Aluminum Company of America.

TABLE 1

Parts Per Million of Aluminum Dissolved By Fruit Juices in Wrought Aluminum Utensils

Fruit Juice	Amt. in Original Juice	1†	2	3	4	5	6	7	8	Average increase in aluminum
Apple	2.2	16.0	19.6	29.3	18.0	20.7	17.0	26.3	26.6	21.7
*Apricot	1.8	3.3	3.7	5.3	5.7	9.2	16.5	15.2	12.5	8.9
Cherry (fresh)	5.3	60.3	51.8	69.3	71.5	61.0	96.3	68.0	88.9	70.9
Grape	3.1	10.3	7.7	10.7	11.3	14.7	26.0	10.7	8.3	12.5
*Grapefruit	4.0	17.6	15.3	17.6	16.0	25.3	36.0	14.3	24.0	20.8
Grapefruit	4.4	8.3	14.7	12.0	15.0	18.3	18.3	16.3	31.6	16.8
*Loganberry	4.4	18.6	20.3	22.0	32.0	52.3	83.0	39.0	60.8	41.0
Orange (fresh)	1.3	4.9	4.4	6.7	10.2	18.0	18.3	12.7	24.0	12.4
*Orange	4.0	7.3	8.3	13.0	11.1	15.7	25.0	15.6	24.5	15.1
Orange	4.3	16.0	10.3	17.6	14.0	20.7	20.3	15.7	16.7	16.4
*Peach	2.0	13.3	11.3	12.0	11.7	11.3	10.7	9.3	10.6	11.3
*Pear	2.0	8.3	10.3	10.3	8.7	6.3	7.7	6.3	5.3	7.9
*Plum	2.7	11.3	10.7	14.7	17.6	15.3	18.7	16.3	25.4	16.3
*Pineapple	4.0	9.7	4.0	9.7	10.7	11.0	18.0	16.3	12.7	11.5
Pineapple	3.6	14.0	13.7	16.0	24.0	26.0	37.6	20.3	29.8	22.7
*Prune	6.2	27.0	33.0	49.0	44.6	36.3	51.9	36.6	54.0	41.6
Rhubarb (fresh)	23.5	102.6	92.3	166.8	197.5	180.5	209.9	200.9	169.6	165.0
Tomato (fresh)	3.1	9.3	10.0	13.0	11.0	20.3	18.6	12.3	16.5	13.9
Yellow Tomato	3.1	21.0	14.0	13.7	31.3	40.0	38.3	55.0	54.0	33.4
Average amount aluminum dissolved from each pan		20.0	18.7	26.8	29.6	31.7	40.4	32.0	36.6	

* Contained added sugar.
† Numbers indicate the different brands of aluminum utensils used.

solved from these two utensils, however, differed considerably.

In the investigation *(13)* in this laboratory on the effect of organic acids on aluminum, it was found that a normal solution of oxalic acid had a decided corrosive action on sheet aluminum. The great corrosive effect of rhubarb noted in the present investigation may be because of the presence of oxalic acid in this food product.

As a rule the presence of sugar in a juice causes less aluminum to be dissolved. This condition was reported by Beal, Unangst, Wigman, and Cox *(1)*. Too much emphasis should not be placed on this observation from the results herein reported, because in all instances, except one, the sweetened and unsweetened juices were from different sources. Ten percent sugar was added to the unsweetened pineapple juice; therefore, with this juice the protective effect of sugar seems to be indicated. Contrary to the usual results, unsweetened grapefruit juice did not remove as much aluminum from the utensil as the sweetened product.

Fresh orange juice dissolved less aluminum than the sweetened canned juice, and the latter juice dissolved less than the unsweetened canned juice. The last two juices, however, were not from the same source, so a comparison between them, perhaps, is not justified. Yellow tomato juice removed larger amounts of aluminum from the utensils than the fresh red tomato juice.

In the cast aluminum utensils (Table 2), as with wrought utensils, rhubarb and cherry juices dissolved the most aluminum; yellow tomato, pineapple, and sweetened orange juices were next in order. Unsweetened grapefruit and pineapple juices both dissolved more aluminum than the sweetened juices of the same fruits. Peach, grape, pear, and apricot juices had less effect on cast aluminum utensils than other juices.

On the average, cast aluminum utensils 2 and 3 were most affected by the fruit juices and utensil 5 was the least affected. On an average with each of the juices, except prune juice, the cast aluminum utensils showed more parts per million of aluminum dissolved than did the wrought aluminum utensils.

Referring to Table 3, it may be seen that the most acid juices are rhubarb, grapefruit, and cherry. The least acid juices are pear and peach. The most acid juice, rhubarb, dissolved the greatest amount of aluminum from the pans; and the least acid juices, pear and peach, dissolved a very small amount of aluminum. The next most acid juice, unsweetened grapefruit, dissolved very little aluminum, occupying tenth place with wrought aluminum utensils and twelfth place with cast aluminum utensils in the amount of aluminum dissolved. Acidity, therefore, is an important factor, but not necessarily the controlling factor, in the action of acid juices on aluminum utensils.

The blank determinations show much more aluminum present in rhubarb than in any of the other foods tested. Prune and cherry juice were next in aluminum content. The amount of aluminum present in the blanks, except with the fresh fruit juices, may be partly due to the preparations of the fruit for canning in aluminum utensils and also to the storage of the juice in tin cans.

Table 4 shows the aluminum content of fresh foods as reported by other investigators *(10)*. The results are expressed as mg. per 100 g. of dried food. Some of the determinations recorded in this table were made as early as 1904, and others date to 1932. There is considerable difference among some of the results. For instance, Berg reports 0.33 mg. of aluminum in apples; whereas Massatsch reports 1.9 mg.; Lehman reports 0.28 mg. of aluminum in oranges; whereas Winter and Bird report 0.94 mg. No doubt these differences were due partly to the methods of analysis used, and the nature of the soils in which the foods were grown. The early methods for the determination of aluminum in food were not so accurate as some of the methods later developed. Beal, Unangst, Wigman, and Cox *(1)* and Bertrand and Levy *(2)* are two of the most recent reports on the analysis of a large number of foods for aluminum.

From Tables 1 and 2 it may be seen that the amounts

TABLE 2

Parts Per Million of Aluminum Dissolved By Fruit Juices in Cast Aluminum Utensils

Fruit Juice	1†	2	3	4	5	Average increase in aluminum
Apple	33.5	57.3	74.1	37.7	19.8	44.5
*Apricot	22.2	23.8	34.2	26.6	20.3	25.4
Cherry (fresh)	143.7	167.7	208.2	103.8	100.2	144.7
Grape	16.3	26.0	18.6	17.5	9.3	17.5
*Grapefruit	26.0	27.8	33.7	30.2	31.6	29.9
Grapefruit	30.4	28.9	38.7	40.4	26.6	33.0
*Loganberry	32.9	59.4	47.4	30.9	58.3	45.8
Orange (fresh)	34.2	57.0	44.7	35.7	65.3	47.4
*Orange	57.1	64.1	57.1	57.7	38.0	54.8
Orange	31.3	22.1	25.2	20.9	44.3	28.8
*Peach	26.4	16.4	11.9	20.0	6.7	16.3
*Pear	24.2	27.2	20.1	26.6	7.7	21.2
*Plum	34.0	34.6	22.2	36.9	29.6	31.5
*Pineapple	34.0	32.6	75.5	49.5	36.0	45.5
Pineapple	64.8	38.4	58.6	85.2	47.6	58.9
*Prune	28.6	70.2	37.3	29.5	23.6	37.8
Rhubarb (fresh)	337.3	336.9	510.6	250.0	262.8	339.5
Tomato (fresh)	35.5	54.6	60.8	36.0	46.0	46.6
Yellow Tomato	67.5	68.8	132.3	103.7	52.0	84.9
Average amount of aluminum dissolved from each pan	56.8	63.9	79.5	54.7	48.7	

* Contained added sugar.
† Numbers indicate the different brands of aluminum utensils used.

TABLE 3

Acidity of Fruit Juices

Fruit Juice	ml. N/10 NaOH to neutralize the acid in 100 ml. juice	Grams acid per 100 ml. of juice as malic acid
Apple	113.3	0.76
*Apricot	61.8	0.41
Cherry (fresh)	164.8	1.10
Grape	92.1	0.62
*Grapefruit	185.4	1.24
Grapefruit	195.7	1.31
*Loganberry	133.9	0.90
Orange (fresh)	154.5	1.04
*Orange	133.9	0.90
Orange	123.6	0.89
*Peach	39.0	0.26
*Pear	20.6	0.14
*Plum	92.1	0.62
*Pineapple	154.5	1.04
Pineapple	154.5	1.04
*Prune	51.5	0.35
Rhubarb (fresh)	236.9	1.59
Tomato (fresh)	82.4	0.55
Yellow Tomato	92.7	0.62

* Contained added sugar.

TABLE 4

*Results of Other Investigators**
(Figures are given in mg. per 100 g. dried substance)

Fruit	Langworthy, and Austen, 1904	Ragnar Berg, 1913	Underhill and Peterman, 1929	Massatsch, 1929	Winter and Bird, 1929	Lehman, 1929-1931	Bertrand and Levy, 1931
Apples	1.3	0.33	0.1-0.53	1.9	0.84	1.8	1.3
Apricots	.....	.....	.....	.....	.....	.....	6.0
Cherries	.....	.....	.....	.....	.....	.....	7.1
Grapefruit	.....	.....	.....	.....	0.75	.....	.....
Oranges	0.45	0.35	0.51	.....	0.94	0.28	0.6
Peaches	.....	.....	.....	.....	.....	.....	6.4
Prunes	.....	.....	.....	.....	2.05	.....	.....
Rhubarb	.....	.....	.....	.....	6.33	.....	3.2
Tomatoes	.....	.....	.....	2.8	.....	3.8	2.2

* Taken from "A select, annotated bibliography on the hygienic aspects of aluminum and aluminum utensils." Mellon Institute of Industrial Research, Bibliographic Series, Bulletin No. 3, Pittsburgh, Pa. (1933).

of aluminum dissolved by foods were very small and below the amounts found by most investigators to have any physiological effect on the body.

Conclusions

1. Fruit juices have less effect on wrought aluminum utensils than on cast aluminum utensils.
2. For the most part, unsweetened juices tend to dissolve more aluminum than sweetened juices.
3. All of the original fruit juices tested contain some aluminum.
4. There is no regularity of action of a given juice on the different brands of aluminum utensils.
5. Rhubarb and cherry juice have a greater effect on both wrought and cast aluminum vessels than any of the other juices tested.
6. The degree of acidity of the juice may be responsible to some extent for the amount of action on aluminum, as shown by rhubarb and cherry juices.
7. The amounts of aluminum dissolved from the different brands of aluminum utensils are small. According to most investigators, such amounts are below those which have any physiological effects on the body.

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MEETING, AMERICAN SOCIETY OF BREWING CHEMISTS

On behalf of the Executive Committee of the American Society of Brewing Chemists, President Philip P. Gray has announced that the next Annual Meeting of the ASBC will be held at New Orleans, Louisiana, April 10 to 14, 1949, with headquarters at the Roosevelt Hotel. The choice of New Orleans is in line with the Society's policy to rotate the annual conventions geographically. At the same time, it is also a recognition of the importance of the brewing industry of the South and its growing membership in the ASBC.

Interesting and diversified activities are being planned for the New Orleans meeting. Under the leadership of Robert I. Tenney, Chairman of the Technical Committee, reports will be given on the progress of the various technical projects. Dr. Eric Kneen has accepted the chairmanship of the Program Committee and the presentation of individual research papers of high caliber is assured. Local arrangements are being made by a committee headed by Ulysses C. Gramsch. Further details will be announced.

VEGETABLE AND FRUIT PROCESSORS' SCHOOL

The Division of Horticultural Products of the Department of Horticulture, The Ohio State University, Columbus, Ohio, has announced the program for the second Vegetable and Fruit Processors' School which will be held at the Horticulture and Forestry Building in Columbus, March 14-16, 1949.

Nine lectures will deal with the subject of food preservation, there will be laboratory exercises covering processing technics and the examination of horticultural products, and there will be periods of discussion in which a wide variety of subjects will be covered.

Food Wastage — A Technology Problem

The problem of food supply is one that involves production and utilization. It should be understood that our food standard is one of the highest in the world and every move should emphasize the need to raise the level in those foods that furnish satisfaction and good nutrition. The problem of food supply is not one that is immeditely pressing in this country. Better processing, preservation, and distribution to prevent loss of nutrients as well as actual loss of food is of equal importance to increased production in keeping a balance between food supply and increased population.

The time will come when our present rate of food wastage due to spoilage in the field, in storage and distribution channels, and in the kitchen cannot be tolerated even in our own country without lowering our standards seriously. What percentage of our supply is wasted is unknown. It is sufficiently large however to offer ample reward for technological developments that will improve conservation. Such conservation may be effected in many ways that are not ordinarily classed as preservation. More convenient packaging should discourage waste in the home. Better storage and better processing may avoid waste of nutrients through deterioration. New developments in harvesting methods will certainly avoid large field losses. These savings are of equal importance with increased production in our race to feed a rapidly growing population.

The world has been slow in solving its food problems, but, for that matter, it has been slow in solving its transportation, communication, clothing, and housing problems. The difference is that people have always recognized food shortage while they had no conception of airplanes, telephones, nylon, and insulated houses, until these things were developed and handed to them. Therefore, one might have expected more attention to the food situation at an earlier date. The plain fact is that developments in all of these fields could not possibly have taken place until man had perfected the experimental method of acquiring accurate information about himself and his environment.

If developments in food have appeared to be somewhat less spectacular than in electronics, let us remember that the human body which utilizes this food is a most intricate system of balanced and coordinated chemical reactions with a fixed relationship. Since we cannot change the machine which uses the food, we must always have products that possess the nutrients in a form not greatly different than that eaten by our ancestors a hundred thousand years ago. While we know much more about the source, the chemical composition, and the function of these nutrients, we have not been able to make any important substitutions. Except for a few of the vitamins which have been synthesized, it may be said that we are dependent today on the same natural sources for our food as were our earliest predecessors. The boundaries that circumscribe the efforts of food scientists are likely to remain until some millions of years may allow the evolution of man into a different physiological specimen.

While the limitation may thwart the ambitious promoter who would like to be the first to provide the astonished world with a square meal in a package no larger than an aspirin tablet, it should also serve as an important guide to the more practical food technologist. Our field is now clearly marked as production, processing, preservation, packaging and distribution of the many long recognized food items in such a manner as to provide the consumer with enough of all the nutrients in palatable form, convenient to use and free of contamination. Meeting these requirements for a world population under the widest possible variation of environment and with each variant under continuous change, but with each consumer a rigidly fixed physiological unit, should be sufficient challenge. These are the problems of the food techcnologist.

R. C. Newton
I. F. T. President 1942-43

New or Old White House?

Since President Truman, in his inaugural address, expressed the idea that "We must embark upon a bold new program of making the benefits of our scientific advances and industrial progress available for the improvement and growth of underdeveloped areas," there has been much discussion of two questions; first, to what extent are we qualified to benefit the underdeveloped areas by transmitting to them knowledge of our scientific advances and industrial progress? second, what accounts for the fact that we are able to show the way technologically to other nations, if we are so qualified?

Many comparisons are being made. A favorite of these involves Great Britain and the United States, although, in such a comparison, we neglect the "underdeveloped areas." In this comparison, we learn that, for research, Britain spends 0.9 percent of its national income, and United States spends 0.6 percent of its national income. These expenditures amount to $6.75 per capita in the United Kingdom and $7.95 per capita in the United States. Since $6.75 will pay for more research in Great Britain than will $7.95 in the United States, it appears that research is at least more intense in Great Britain than in the United States. One may state that results seem to reflect the logical expectation from that situation—that Great Britain leads the United States in the production of fundamental scientific knowledge.

In admitting this fact, we have not answered the question of to what extent the United States is qualified to help other nations technologically. Certainly, if the United States is qualified to help Great Britain, one of the world's most advanced nations, it is more than qualified to help a "backward" nation, as Mr. Truman suggests. To answer the question, we may simply say that it is generally admitted that technology in the United States is ahead of technology in any other country. That seems to be a fairly satisfactory answer to question No. 1.

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